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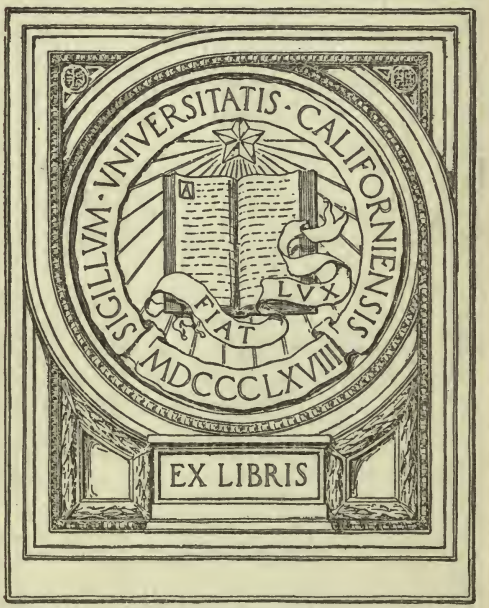

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A MANUAL

OF

CHEMICAL PHYSIOLOGY.

A MANUAL

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CHEMICAL PHYSIOLOGY

INCLUDING

ITS POINTS OF CONTACT WITH PATHOLOGY.

BY

J. L. W. THUDICHUM, M.D.

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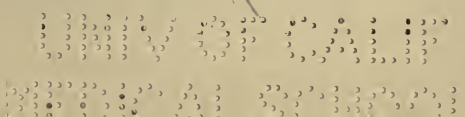

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PREFACE.

The first part of this little treatise was written and printed as the introduction to my "Researches intended to promote an Improved Chemical Identification of Diseases," which have been published in several numbers of the annual "Report of the Medical Officer of the Privy Council." It has been found so useful in my experience as a teacher, that I have, with the permission of Mr. Simon, ventured to reproduce it in the present form. It is a complete but concise epitome of the branch of science commonly termed "physiological or animal chemistry," and will be found to contain its latest acquisitions. Any medical student who possesses the information which it contains will be enabled to meet the requirements, so far as concerns this particular subject, of any of the examining and licensing bodies in this country and abroad. To the student in Chemistry, Physiology, or Science, it offers a ready help to the acquisition of elementary knowledge, upon the basis of which he can afterwards place the superstructure of more extended and detailed

13555

studies. To my colleagues of the Medical Profession it will afford an easy bird's-eye view of the chemical features of the field of their thoughts and action. Its perusal will involve no unreasonable tax upon the time of any reader or student, and occasional reference to particular points is facilitated by marginal notes and a short alphabetical index.

The second part of the work is an *Analytical Guide* for the use of those who desire to make themselves practically acquainted with the phenomena and constituents of animal bodies. It is therefore not descriptive in the sense in which ordinary chemical text-books may be said to be so, but *prescriptive* in the style and manner of pharmacopœias. It directs the student how to proceed in order to arrive at a certain result, leaving him in most cases to appreciate the result of his operation by his own reflection. The guide is perhaps the most elementary that could be written for any practical purpose, and yet I think it improbable that ordinary students of medicine will easily go through the whole of its matter in the laboratory. I hope, therefore, that teachers of chemistry who will make use of the Guide in their classes will select the reactions and analyses to be performed by each student according to his knowledge, ability, and intentions.

This little treatise summarises much of the method

pursued, and many of the results arrived at in my laboratory during many years of patient inquiry. In the compilation of the Analytical Guide I have received much valuable help from my esteemed assistants, Mr. F. J. M. Page, and Mr. C. G. Stewart, for which I here express to them my sincere thanks.

THE AUTHOR.

3, Pembroke Road, Kensington, W.;
April, 1872.

CHEMICAL PHYSIOLOGY,

AND

ITS POINTS OF CONTACT WITH PATHOLOGY.

Digestion.

THE food of man, variously prepared by mechanical processes and chemical operations (cooking), is comminuted in the mouth by chewing. At the same time it is mixed with a variety of fluids, some of which have chemical powers and predispose the food to a change, while others serve mechanical objects only. The mixture of these fluids is termed saliva; Saliva. but however homogeneous may appear that mixture, the properties of its components are very various. For the secretion of every particular kind of glands, of which there are four, differs, and the secretion of one and the same gland or set of glands may vary according to the agencies which call them into action. Underneath the forepart of the tongue is secreted from one and the same duct the saliva of a gland which lies under the tongue (sublingual), and that of two other glands which lie farther back on both sides of the tongue underneath the lower jaw (submaxillary glands). To collect either of these secretions little

tubes have to be carefully introduced into the respective ducts, which is a matter of some difficulty. For this reason the chemical composition of the secretions of the separate glands is very imperfectly known. Experiments upon animals have shown that these glands can give four different kinds of secretion, according to the nerves which are irritated for the purpose. One nerve, a branch of the facial, and a continuation of the chord of the tympanum, on irritation causes a clear, slightly ropy secretion from the submaxillary glands.

Chordal Saliva.

This "chordal" saliva contains about 4 per cent. of solid matters, of which 1·5 are globuline, mucine, and coagulable albumen; 2·5 per cent. are mineral, mainly alkaline chlorides and lime-salts; of these latter the carbonate, dissolved in excess of carbonic acid, frequently decomposes in the mouth, and deposits crusts of lime carbonate upon the teeth, which are popularly called tartar.

Sympathetic Saliva.

On irritation of the sympathetic nerve the submaxillary glands secrete an opaque very tough saliva. This contains from 15 to 28 per mille of solids, amongst which is mucine, and granules or roundish lumps of an albuminous matter, and much free alkali.

Ganglionic saliva.

The third kind of saliva is that which flows when the submaxillary ganglion is made the centre of a reflex action which works by way of the lingual nerve. This is the only secretory act without the intervention of cerebro-spinal influence that is known at present.

Paralytic saliva.

The fourth kind of saliva is the "paralytic" or thin watery fluid which is secreted under the influence of nervous paralysis, caused either by degeneration, or poisoning, or wounds which separate

the secretory nerves. Its composition is not yet ascertained.

Rhodanates in saliva.

The mixture of sublingual and submaxillary saliva in man (not in animals) contains rhodanate or sulphocyanate (also termed rhodanide or sulphocyanide) of potassium and sodium C N K S, and C N Na S, recognised by the red colour which iron-chloride imparts to saliva, or to the distillate obtained from it with acids. This phenomenon admits at present of no particular theory.

Parotid saliva.

The saliva which is secreted by the parotid glands can easily be collected by the introduction of canulæ into the ducts. It is an alkaline, hardly viscous fluid, which contains a little albumen, some globuline, a particular ferment termed ptyaline, but no mucine. It contains much rhodanate, and is the most suitable material for preparing the distillate of rhodanic acid. It contains, water 995·3; solids 4·7; of these are organic 1·4; mineral 3·3; of the latter there is lime carbonate 1·2. The parotid saliva transforms starch into sugar by means of the ferment termed ptyaline. This is the only agent in saliva which has that power. It can be isolated by adding phosphoric acid and subsequently lime to saliva. Ptyaline adheres to the phosphate, is washed out by water, and precipitated by alcohol. It contains nitrogen but is not albuminous, refusing to yield the xanthoproteic acid reaction with nitric acid. The diastase of malt has a similar action used in trade fermentations. An interesting and important application has lately been made of diastase by Baron Liebig, for the production of a food for infants, which supplies

Ptyaline Glycogeny.

Diastase. Liebig's food.

efficiently the want of alkali and ptyaline in the digestive juices of children who are being brought up without mother's milk, or with such as is not in a healthy state. Diastase acts best at 66° C, while ptyaline is destroyed at 60° C. An agent similar to ptyaline is

Emulsine or synaptase.

emulsine, or synaptase of almonds, which has been recommended as a dietetic remedy in diabetes. But it does not seem to affect starch in any way, although decomposing amygdaline and salicine.

Amygdaline. Water. Oil of bitter almonds. Prussic acid. Sugar.

$$\overbrace{C_{20}H_{27}NO_{11}} + \overbrace{2H_2O} = \overbrace{C_7H_6O} + \overbrace{CHN} + \overbrace{C_6H_{12}O_6}$$

Salicine. Water. Saligenine. Sugar.

$$\overbrace{C_{13}H_{18}O_7} + \overbrace{H_2O} = \overbrace{C_7H_8O_2} + \overbrace{C_6H_{12}O_6}$$

In these transformations sugar is a collateral product, while in that of starch by ptyaline or diastase it is the only product.

Starch.

Starch consists of two bodies, which in the little granules are disposed in alternating layers. The first is granulose, and has the property of being coloured blue at once by free iodine. The second is cellulose, not coloured blue by iodine at once, but only after sulphuric acid or zinc-chloride has been allowed to act upon it. When unboiled starch is mixed and digested with saliva for days, the granulose is dissolved out of the corpuscles and transformed into dextrine and sugar, and the cellulose only is left. At higher temperatures this also is changed. Boiled starch is more easily transformed, as the granules are burst and admit the altering juices between their layers with

facility. The first symptom of the addition of saliva to boiled and cooled (to 40° C)starch or pap is increased fluidity, indicating the formation of soluble starch and dextrine. At a later period only sugar is formed, according to the following formulæ :

Starch (synonym = amylon) . . . $C_6 H_{10} O_5$

Soluble starch . . } The same formula as starch.
Dextrine . . . . }

$$\overbrace{C_6H_{10}O_5}^{\text{Dextrine.}} + \overbrace{H_2O}^{\text{Water.}} = \overbrace{C_6H_{12}O_6}^{\text{Sugar.}}$$

Amongst the products of degeneration of the spinal marrow in locomotor ataxia, there are bodies resembling starch corpuscles, but consisting entirely of cellulose. Amyloid degeneration.

I had an opportunity afforded me of examining such a case, in which the sense which in physiology we now term the sense of pressure was almost entirely lost, while the sense of pain and the sense of heat and cold persisted. On examining microscopically, and by means of sulphuric acid and iodine, thin sections of the spinal marrow, I found in various parts of it agglomerations of blue bodies imitating in a remarkable manner wheat-starch coloured blue by iodine. The term *amyloid* is perfectly correct as applied to this particular degeneration. The reaction of these bodies, however like to that of amylum, is not that of amylum itself, for the latter is coloured blue by iodine alone, while these bodies require the concurrence of sulphuric acid and iodine.

The secretion of the salivary glands mixed with the mucus secreted by little follicles, in the mem- Mixed saliva.

branes of the cavity of the mouth, constitutes mixed saliva. This can be collected in quantity by irritating the fauces with a feather, and producing vomituritions. It does not reduce alkaline copper solution, but retains a little copper oxyde in solution when cupric salt and alkali only are added. It transforms starch into sugar, so that chewed pap after some standing, with cupric sulphate and caustic potash, at 70° C, yields red copper suboxyde. It does not change cane-sugar into invert sugar, and thus differs from yeast. The quantity of mixed saliva secreted by a man in 24 hours varies between 300 and 1500 grammes; it may be greatly increased by excitants, and irritating medicines and poisons.

Saliva in disease.

Little is known of saliva in disease, but the investigations of the future promise further results. In diseases, such as salivation under the influence of mercury, rhodanates disappear. The saliva then contains mercury. Many medicinal salts pass easily into the saliva from the blood, such as iodide and chlorate of potassium, and when used long in quantity produce slight salivation. In diabetes the saliva contains lactates, but no sugar. In the paralytic saliva of hysteric persons leucine has been found. Acid saliva seems to contain lactic acid, and is of course anomalous. The presence of urea has been alleged, but not proved with certainty. In hydrophobia the saliva is the bearer of the contact-poison by which the disease is propagated to other individuals.

Anomalous ingredients.

Digestion of starch.

While the saliva influences starch as indicated, and does not lose its action by the admixture of acid of

the concentration of the gastric juice, it certainly, under ordinary circumstances, does not transform the whole of the starch into sugar. The gastric juice has no influence on starch; the pancreatic juice a trifling influence in the same sense as saliva. Deducting all sugar and lactic acid to be met with, it is necessary to assume that other products are formed, which yet elude analysis. Do any of these products find their way into the liver, and are these transformed into glycogen?

Glycogen of the liver.

Glycogen is a kind of dextrine, which was discovered in the liver by Bernard and Hensen. It occurs in three forms, of which one of the formula $C_6H_{10}O_5$, is powdery, two others, $C_6H_{12}O_6$, and $C_6H_{14}O_7$, are gummy. It polarises to the right four times more intensely than dextrose sugar. With a solution of iodine in iodide it gives a dark red colour. It dissolves copper oxyde without reducing it. By sulphuric and hydrochloric acids, saliva, pancreas juice, serum of blood, and cold prepared extract of liver, it is transformed into dextrine and ultimately into sugar. Many physiologists have endeavoured to explain the source and destination of this matter, but as yet without any very complete success. Regarding its origin, it has been found that it could not be formed from sugar, as the portal blood did not contain any. It was not formed from fats. Animal food enabled animals to form it, whence the conclusion was drawn that glycogen originated in albumen. Seeing that the liver decomposes albumen, as proved by the constitution of the bile, this idea has much in its favour, but the experiments upon which it is based admit of different interpretation. Muscle

frequently contains dextrine, always inosite (a particular kind of sugar), and lactic acid. All these might enable the liver of the animal which eats the flesh to form glycogen. At present it is uncertain from which material the liver forms glycogen; possibly it is formed out of starchy and albuminous matters at the same time; at least most of it is formed (up to 12 per cent. of the weight of the liver in fowls) when these two kinds of food are digested together in large quantity.

Destiny of glycogen.

As the dead liver was found to transform glycogen quickly into sugar, and as some sugar could be found in hepatic blood, it was concluded that glycogen is transformed into sugar, and passes into the blood, to be there oxydised or changed as required. This view, upon which was based an entire theory, called that of the glycogenetic function of the liver, was received for some years by physiologists in general, until one of its greatest admirers, Pavy, believed that he had discovered it to be erroneous. According to him no sugar is made in the liver in the living healthy body. I showed that his experiments admitted of such variation as to prove either his or Bernard's doctrine. At present the bulk of evidence goes to show that, as a portion only of the starch in the intestines is transformed into sugar and passes into the chyle, so a portion only of the glycogen of the liver is transformed into sugar and passes into the blood. Quantitative experiments on a large scale, combined with the chemolytic method of research, will alone be able finally to decide the matters under discussion.

Mal-assimilation of starch.

When sugar in considerable quantity exists in the

blood, the body cannot deal with it, and excretes the sugar unchanged. Such a condition constitutes the disease termed diabetes, which appears to be a much more complicated disease than its main symptom taken alone would seem to indicate. The oxydation of sugar only is diminished or not accomplished, that of the albuminous substance and fats is rather increased, sometimes enormously so; therefore the carrying power of the blood-corpuscles for oxygen cannot be diminished as has been supposed lately, at least not in all cases of diabetes. There must be a perversion of chemical agency, as proved by the appearance of lactic acid in the saliva and of acetone in the stomach and the urine. On the whole there is at present neither a plausible theory nor a rational treatment of diabetes, as evidenced by the fact that noted physicians now maintain that diabetic patients eating promiscuously everything are better off than patients who abstain from starch and confine themselves to the anamylic diet so elaborately prescribed by Bouchardat. The sugar may be made in the liver or in the muscles, it may be the effect of a change of nervous influence (as suggested by the artificial diabetes of animals after wounds of the fourth ventricle of the brain), or of a failure in the supply of a ferment capable of transferring oxygen to it. The sugar when once in the diabetic blood appears not to be increased or decreased by standing of the drawn blood out of the body, the blood consequently contains perhaps no glycogen. This was ascertained by a special experiment, made upon the blood of a diabetic patient.

Diabetes.

Digestion of the food in the stomach.

The comminuted food mixed with saliva arrives in the stomach and excites this organ to a mechanical and chemical action, termed digestion. The many little rennet glands situated in the walls of the stomach

Gastric juice.

secrete a liquid termed the gastric juice, which in man contains 994·6 per mille of water and 5·39 of solid and permanently fluid ingredients other than water. Of

Pepsine.

these 3·0 are pepsine, 0·2 hydrochloric acid, with which perhaps a small quantity of lactic acid is mixed, and chlorides of the alkalies, with some phosphates of earths. Singular is the presence of some calcium-

Gastric fistula.

chloride in the juice. The juice has been examined mainly as obtained from persons who by accident had fistulous openings in their stomachs, and upon dogs upon whom such fistulas had been formed by operative interference. This led to the formation of artificial juice, which requires the addition of natural pepsine, and is therefore only in part artificial. It serves, however, for the purpose of studying stomach digestion upon many kinds of food, and of supplying a kind of remedy in diseased conditions in which the natural juice is supposed to be deficient.

Power of gastric juice.

This gastric juice possesses the power of dissolving or reducing to a liquid state albuminous substances, which are either by preparation, such as boiling, or by nature, insoluble in water. Albumen, caseine, fibrine, syntonine, the albuminous substances of vegetables, gluten, and the collagene tissues or gristle, are under the influence of gastric juice, or of a mixture of pepsine and hydrochloric acid, dissolved to thickish

somewhat turbid matters, to which the name of peptones is given. Pepsine may be isolated by mechanical precipitation in the same manner as ptyaline by adhesion to phosphate of lime. It is not itself destroyed during digestion, but is capable of transforming great quantities of solids into fluid by that mysterious influence termed contact action. When the juice is saturated with peptones it ceases to act, but an addition of dilute acid fluid enables digestion of newly introduced albuminous matters to be effected. The secretion of the hydrochloric (and lactic ?) acid from the stomach glands is a chemolytic process by which salts of alkalies are split up into acid and base. Of this action I shall show the completion of the circle in the biliary function immediately to be described. The origin of the pepsine is the blood, but which ingredient of this fluid yields this curious substance, which is so different from albumen, cannot be told. In the stomach digestion saliva by its ptyaline forms some sugar, the gastric juice fluidifies the albuminous matters, the fats are made fluid and liberated from their tissue connections, vegetable structures are variously disintegrated, and the whole is mixed with water and a small amount of air carried down in the process of swallowing. Other decompositions, as yet imperfectly understood, also take place, as evidenced by the strong odour of the digested matters, and at last the homogeneous mixture of substances, termed chyme, passes through the pylorus into the duodenum.

Peptones.

The ingredients of chyme are starch, sugar, fat, and peptones, or if only animal food had been eaten, fat

Chyme.

Peptones.

and peptones alone. There are also undigested pieces of flesh, albumen, caseine, constantly present. It is at present impossible to say what these albuminous matters are. Some physiologists say there is only one substance, others that there are five and more, the statements as well as the experiments upon which they are based being quite irreconcileable with each other. None of these researches have as yet been carried out by means of the quantitative chemical method, excepting the comparison of the composition of the peptones with the original matters. It was found to be almost unchanged. The peptone solutions are not coagulated by boiling, but are precipitated by absolute alcohol. They give Millon's reaction with nitrate and nitrite of mercury. They diffuse easily through parchment paper (dialyse) into water, exhibiting a property towards membranes of the utmost importance for absorption, which albumen possesses only in the very slightest degree. Optically they are characterised by turning the plane of polarisation towards the left.

The coagulation of milk in the stomach, or by rennet out of it, is supposed by some to be due not to pepsine, but to another ferment which transforms sugar of milk or lactose into lactic acid, and precipitates the soda-albumen or caseine. This matter is problematical.

The quantity of gastric juice secreted daily in the human stomach has been estimated at 10 per cent. of the body-weight, or 16 lbs.; other direct observations, however, lead to 30 lbs.

Gases.

During digestion some gases, consisting of carbonic acid, hydrogen, and nitrogen, are not rarely formed from the digesting food. This may become a distressing symptom in disease.

Duodenal digestion.

Duodenal digestion is a continuation of stomach digestion under greatly complicated circumstances, since the chyme receives additions of bile and pancreatic juice. The physiology of these liquids has been studied upon fistulous openings occurring accidentally in man, or produced by art in animals. The secretory acts and influences are no doubt well known, particularly their variations under several conditions. But the employment of the secreted matters is by no means so elucidated as to be capable of satisfactory theoretical representation. The pancreatic juice has probably three functions, of which one is the completion of the solution of the pieces of meat and albumen which issue from the stomach with the chyme; another is the decomposition of fat into glycerine and fatty acid; and a third the emulging of neutral fat, and the transforming of it into a subdivided condition, in which it may pass through the pores of the mucous membrane into the chyle-ducts. It also transforms a small quantity of starch into sugar. These properties are only possessed entire by juice which is abstracted from the pancreatic duct of an animal during full digestion, or from a reddened pancreas. Juice thus procured is tough or viscid, and contains 10 to 11 per cent. of solids, while juice obtained from a permanent fistula has only 5 per cent. of solids, and lacks the power of digesting albuminous fragments. It is probable that

Pancreatic juice. Three functions.

this deficiency is caused by a degeneration of the gland consequent upon the operative interference. The juice contains an albuminous matter at present undefined, possibly some mucine, and generally leucine, which is present in the parenchyma of the gland in larger quantities: it has a more or less alkaline reaction.

Biliary functions.

The liver has an obvious function, and that is to secrete bile. It seems almost superfluous to make such a statement, but the views of physiologists regarding this organ have so often been perverted that it is necessary to recur to elementary principles. The error regarding the function of the liver which has crept into physiology has mainly been caused by the discovery in it of a substance which has the capability of being transformed into sugar, namely, the above-described glycogen, also called hepatine, or liver-dextrine; and in consequence of that in itself remarkable and interesting discovery it has generally been believed that the main function of the liver was that of forming sugar. We know now that such is not the case.* The main function of the liver is one of considerable intricacy, and essentially connected with the great features of the process of digestion.

Connexion of stomach digestion with the bile function.

Digestion in the stomach is produced by a process in which of chemical ingredients hydrochloric acid

* See the articles of Pavy on this subject, 'Guy's Hosp. Rep.,' 1858, iv, p. 291; 'Phil. Trans.,' 1860, p. 595, and my critical experiments in 'Brit. Med. Journ.,' vol. i, 1860. In these latter the analytical method now generally followed was first used and published; it was afterwards adopted by Pavy, Bernard, Kühne, and others. For confirmation of the variable results see Meissner, 'Jahresbericht für,' 1862, p. 310 *et seq.*; Ritter, 'Zeitschr. f. rat. Med.,' 24, 65; Eulenburg, 'Journ. für Pract. Chem.,' 103, 108, 1868.

takes a main part. In the dog this hydrochloric acid is so strong that the hardest bones are absolutely dissolved. In man such a solution of bones cannot easily take place, but they are certainly corroded when introduced into the stomach. The acid which dissolves them in the dog is hydrochloric acid only; in man it is probably a mixture of hydrochloric and lactic acid. But although we find in the economy chlorides everywhere, and lactates constantly in the chyle, yet we do not meet with these acids in the free state. We are therefore obliged to assume that in the walls of the stomach a chemical process is constantly taking place by which hydrochloric and lactic acids are formed. This process is very simple, consisting in the separation of the chlorine from sodium chloride (or common salt), and the combination with it of a certain quantity of hydrogen derived from the water. What takes place in the glands of the stomach may therefore be stated to be a splitting-up of water and sodium chloride, and a cross combination of the elements to hydrochloric acid on the one side and sodium hydroxyde on the other.

$$\underbrace{H_2O}_{\text{Water.}} + \underbrace{NaCl}_{\text{Common Salt.}} = \underbrace{HCl}_{\text{Hydrochloric acid.}} + \underbrace{NaHO}_{\text{Sod. hydroxyde.}}$$

The lactic acid is produced from lactates in a similar manner, and in the formula of its formation the place of chlorine in the foregoing formula would be occupied by the formula $C_3H_5O_3$. This is simple and certain. But we find in the body no caustic soda,

or sodium hydroxyde, and we are therefore driven to inquire what becomes of it when so produced.

Why the stomach does not digest itself.

Before it is carried away from the gastric glands by the blood, a part of this sodium hydroxyde has an important function to perform, namely, to protect the stomach against the corrosive action of its own secretion.* It keeps the blood and tissues more alkaline, and prevents the acids and pepsine, which have become more energetic after secretion and mixture with the peptones, from corroding the texture of the stomach. (Such corrosion immediately takes place in cases where the supply of blood to a part of the stomach is interrupted, or where food remains in the stomach undigested after the secretory energy has passed away; gastric ulcer, hematemesis, chronic dyspepsia, or painful digestion with follicular erosion, and other pathological conditions, are produced in this way.) The sodium hydroxyde is soon transformed into carbonate in the blood, and passes through the gastric veins into the portal, and thus into the liver. When

Bile.

we analyse bile and add to it a quantity of acid, we precipitate certain matters, namely, the biliary acids formed by the liver, and the cholophæine; and if we evaporate the liquid we get a large quantity of sodium combined with the acid which we have added. Supposing now we take bile and add to it hydrochloric acid, we find at the conclusion of our experiment that sodium has been combining with chlorine and that we

* This function was clearly developed and stated by me in 'Brit. and For. Med.-Chir. Review,' October, 1861, p. 429. At a later period it was made the theme of some interesting experimental inquiries by Pavy ('Phil. Trans.,' 1863).

have sodium chloride, and that the hydrogen of the acid has again combined with oxygen (hydroxyl) and formed water.

$$NaHO + HCl = NaCl + H_2O$$

That is to say, by boiling bile with hydrochloric acid we reproduce the chloride of sodium which before has been decomposed in the walls of the stomach. In our food we take no salt of sodium combined with biliary acid, or any acid that can be transformed into it. We take many substances containing sulphur and nitrogen which can furnish the biliary or special organic part of the bile, but not the soda salts contained in it. Their production is just the function of the liver. The liver splits up or chemolyses albuminous substances or albumen into products of which a part is at present unknown, another part, however, well known under the name of biliary acids and coloured ingredients. They are taurocholic acid, glykocholic acid, cholophæine, bilifuscine, biliprasine, choline, lecithine, and cholesterine.

Taurocholic acid ($C_{26}H_{45}NO_7S$) contains all the sulphur of the bile. By this ingredient it manifests itself as a derivate of albumen, which also contains sulphur. Glykocholic acid ($C_{26}H_{43}NO_6$) is nitrogenous only, and free from sulphur. Both acids yield by chemylosis an acid free from nitrogen and sulphur, cholic acid ($C_{24}H_{40}O_5$). But the sulphuretted acid yields as the second product a body containing all the sulphur and nitrogen of the original acid, namely, taurine= $C_2H_7NO_3S$ = dehydrated isæthionate of ammonia, and

producible from such; while the other compound acid yields as the second product of its cleavage glykokoll or amido-acetic acid ($C_2H_5NO_2$), which is producible artificially by various processes. The rational constitution of the smaller nuclei is thus shown to be well known; but the same could not be said of cholic acid. Glykokoll appears in an excretion as hippuric acid (in which it is coupled with benzoic) but it is at present uncertain whether this excreted glykokoll has previously taken any share in the composition of bile or not. Taurine, however, is consumed in the body, and its sulphur appears in the excretion as sulphuric acid.

The coloured ingredients of bile are cholophæine or bilirubine, $C_9H_9NO_2$, and bilifuscine, probably $C_9H_{11}NO_3$. By oxydation and loss of carbonic acid cholophæine easily passes into biliverdine, $C_8H_9NO_2$, according to the formula, $C_9H_9NO_2 + 2O = C_8H_9NO_2 + CO_2$.

Cholesterine ($C_{26}H_{44}O$) occurring in the brain and blood is no doubt excreted by means of the bile. It is a polydynamic alcohol, capable of forming ethers analogous to fats. Coloured matters, such as cholonematine, boviprasine, fuscopittine, muscoprasine, and ethochlorine, all possessing characteristic properties and spectra, and cholesterine are the main residues of certain diseased processes which terminate in the production of calculi. In a degeneration of the liver, called bacony, considerable quantities of cholesterine remain stagnant in the parenchyma of that organ. The choline of the bile is an organic base of

the composition $C_5H_{15}NO_2$. It is closely related to neurine, $C_5H_{13}N$, a base obtainable, together with choline, from cerebric acid or lecithine, and we are justified in assuming that it is derived from the decomposition of that body. Lecithine consequently may be considered as a normal ingredient of bile.

Pettenkofer's test.

The biliary acids yield a particular test, called, after its discoverer, Pettenkofer's reaction. When mixed with sugar and sulphuric acid they produce a splendid purple colour. It has been found that other acids, such as lithofellic, also yield this test; and I found, further, that cerebric acid yielded it with rare intensity. I therefore applied the spectroscope, and was glad to discover some means for the distinction of the various acids in the coloured test solution. The biliary acids show two bands, cerebric acid and vitelline only one. These spectra are, however, difficult to observe, and require sunlight or oxhydrogen light for their complete development.

Quantity of bile in man.

The quantity of bile secreted in the human body in a day has been estimated at 1200 grammes, or the bulk which would fill a wine bottle and a half. Conclusions from quantities observed in animals can only be used with caution, as some animals, *e. g.*, the guinea-pig, produce enormous quantities of bile relatively to their body weight, while dogs and sheep produce relatively small quantities. The production is more likely to stand in proportion to the size and weight of the liver (hitherto neglected as a physiological factor) than to the weight of the body, to which hitherto quantities were almost exclusively referred.

The bile in the intestine.

The function of the bile is evidently like its chemical constitution, very complicated. Stored during intervals between digestion, it is mainly secreted as well as expelled from the gall bladder during digestion, and a particular quantity of it during a peculiar episode at the end of the emptying of the stomach. On being mixed with the acid of the chyme, the biliary acids are set free, but not precipitated, as the soluble taurocholic acid holds the glykocholic in solution. But a precipitate of peptones is nevertheless produced in the mixture of chyme and bile. This, mixed with the bile-acids and the biliary colouring matter, passes along the intestine as a resinous adhesive substance, to be altered and made absorbable by the many influences of intestinal reaction. It is soluble in alkali, and as much of the intestinal secretion besides gastric juice is alkaline, the transformation meets with no difficulty. The peptone then may pass into blood and chyle as albumen and fibrinogen and fibrinoplastic matter, but the bile is not so easily accounted for. The acids certainly split up, taurine and glykokoll returning into the circulation, but the cholic acid mainly disappears, without leaving any trace in the blood or chyle; neither contain a trace of biliary matter. In the fæces only occurs a small proportion of the cholic acid, amounting in man to from two to three grammes, being perhaps one eighth or one twelfth of the entire amount secreted. The cholophæine has been also changed and become insoluble in chloroform. We must therefore assume that the cholic acid is already split up or chemolysed in the intestine, and reaches

Fate of peptones.

Changes of bile.

the circulation in the more simple form of products of its decomposition. The bile influences fats and fatty acids in the manner of a soap. It communicates to the small absorbing tubes an attraction for fat, so that capillarity raises the fat to a higher point than the same vessel would do without bile. Bile then represents the accomplishment of a purpose which we term chemolysis of albumen. But the further uses of bile are numerous and important; the excretion of cholesterine, intimately related to the chemistry of the nerves, and possibly a product of their action; the excretion of choline and probably lecithine, of which the objects are continuous; the excretion of cholophæine, of which the objects are at least obscure. Bile precipitates pepsine, and when it regurgitates into the stomach arrests digestion completely. It therefore puts an end to pepsine digestion in the duodenum, and favours the alkaline pancreas-digestion. In disease the bile may be retained and cause jaundice, and slowness of the pulse; or it may be decomposed in a peculiar mannner and produce concretions; in man these consist of cholesterine and modified bile-acid, and bilifuscine, with cholophæine and earths; in oxen the cholophæinate of lime predominates, and modified bile-acids with lime-soaps are in lesser quantity. In pigs these calculi contain a peculiar lime salt which assumes a voluminous crystalline form, when the powder is digested with cold alcohol. The pancreas has in diseases been observed to be degenerated and cancerous; and as in these cases lumps of fat are stated to have been observed in the fæces, this ap-

Bile in diseases.

Pancreatic juice in diseases.

pearance of fat has been ascribed to the failure in the supply of pancreas-juice. In lientery the pancreas as well as the stomach and pylorus is probably at fault.

Digestion in the small intestine.

The digestion in the small intestine is very imperfectly known, and requires particular and great researches in the future. In this part of the body is the constant and principal seat of diseased processes of the greatest importance, *e. g.*, typhus and typhoid fever, cholera, and others, not the least important amongst them the æstival and autumnal diarrhœa so fatal to many persons in every year without special epidemic influences.

Fats of the human and animal body.

Glycerine is a tridynamic alcohol, constructed according to the type of water thrice condensed. In this type there are six atoms of hydrogen replaceable; when three of these are replaced by the tridynamic radical glyceryl, glycerine is formed. Glycerine, therefore, has three atoms of typical hydrogen, which can be replaced by three atoms of a monodynamic body or one atom of a tridynamic body. It is not necessary to substitute all the hydrogen at once when we work synthetically, but in animal bodies all the hydrogen is always replaced by fatty acids. A fat is thus shown to be a body of the type of three atoms of water condensed to one, thus—

$$\left.\begin{matrix} H_3 \\ H_3 \end{matrix}\right\} O_3$$

in which three atoms of hydrogen are replaced by one atom of glyceryl^{III} and three others by three atoms of

fatty acid radical.[1] Tributyrine, a fat occurring in butter, has this formula:

$$\left.\begin{array}{l} 3 \text{ monodynamic atoms of butyryl} = (C_4H_7O)^{I} \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad (C_4H_7O)^{I} \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad (C_4H_7O)^{I} \\ 1 \text{ tridynamic atom of glyceryl} \quad = (C_3H_5)^{III} \end{array}\right\} O_3$$

held together by three didynamic atoms of typical oxygen.

The Roman numeral to the right of the radical butyryl signifies that it is a monodynamic radical, and can replace only one atom of hydrogen; but the Roman numeral to the right of glyceryl shows it to be a tridynamic radical, which by its one atom replaces three atoms of hydrogen.

The other fats occurring in the human body and in animal food are tripalmitine—

$$\left.\begin{array}{l} 3(C_{16}H_{31}O) \\ (C_3H_5)^{III} \end{array}\right\} O_3$$

which is equal to the large formula of

$$C_{51}H_{98}O_6,$$

tristearine, a fat abounding in mutton and beef, of the formula

$$C_{57}H_{110}O_6$$

and trieleine, a more fluid fat, which abounds in the oils extracted from lower animals and vegetable seeds.

Yellow animal fats contain luteine, a yellow coloured substance showing peculiar absorption phenomena (three bands) in the blue part of the solar spectrum.

When these fats have passed through the stomach, Digestion of fats.

and after solution of all tissue by which they are held together in the parts of animals, arrive as fluid oils in the intestine, they are acted upon by the pancreatic juice and the bile. The former affects them in two ways. A small portion it decomposes, so that glycerine and fatty acid are formed, another portion it emulges, or makes fit to become subdivided into the very minutest mechanical molecules, and in that state to pass through the small pores of the cylindric cells which cover the villi of the intestine. The fatty acids mostly combine with soda and pass as soaps into the venous blood, and with this to the liver; while the emulged neutral fats pass into the lymphducts, and by them into the venous blood, without passing through the liver. After incorporation with the blood the fat is burned up in the system, particularly the muscles.

Phosphatic emulsion of stearic and palmitic acids.

But there is yet a particular form in which fatty acid is found in the body, namely, emulged in phosphate of sodium solution. When palmitic or stearic acid is boiled with common sodium phosphate, it forms a milky fluid; the acid is so finely subdivided that the microscope can distinguish only the very finest molecules. When this process is applied to neutral fats, or to oleic acid, no effect is produced. The emulsion is a very loose combination, inasmuch as ether extracts the fatty acids. This particular form of fatty acid emulsion occurs in lipohæmia, or fatty blood disease, in chylous urine, and in several effusions into internal cavities.* Its formation is a normal occurrence, the phosphate of sodium of the food (bread) yielding the

* Compare on this subject my researches published in the 'Lancet.'

mineral ingredients for the carrying on of the process with the digested fat in the intestinal canal; if the phosphate be wanting in the food, the bile which always contains notable quantities, will supply it [and it is to this action of the phosphate in bile that any influence upon fatty acids which it possesses is due, the action upon neutral fats formerly ascribed to it being an erroneous conception]. The anomaly in the above-mentioned diseases is the persistence of the fatty emulsion in the arterial blood, whereby an obstruction of the circulation and consequent effusion (of blood, serum, fatty and fibrinous serum, as in apoplexy, dropsy, chylous urine, and other diseases) is produced.

Fatty degeneration.

Fat having been frequently found in degenerating tissues, deposited in a visible manner, in parts where healthy structure shows no visible fat, microscopic anatomists admitted a particular fatty degeneration, in which fat in excess assumed the place of albuminous matters. The heart was supposed to be particularly subject to this disease, to which, however, all other tissues paid tribute. This doctrine, however, is at present in a very unsatisfactory state, and requires much elucidation by researches conducted upon mathematical principles. That fatty degeneration so called may be a very complicated chemical disorder, I showed many years ago by the demonstration of changes in the myochrome of the muscles of the heart, which produced a green granular pigment.*

Chyle

Chyle is the fluid which the lymphatic vessels of the

* See 'Trans. of the Path. Soc.,' vol. vi, 1856, p. 141, and 'Quart. Mic. Journ.,' vol. iv, 1856, p. 111.

villi of the intestine absorb from the digested food and carry to the blood. It contains much fat, to which it owes its white milky appearance. It further contains the ingredients for the formation of fibrine, and curdles soon after it is withdrawn from circulation. Then there is potassium-albumen, and the ordinary albumen of serum. There are also lactates, sugar, and urea contained in chyle, besides a certain amount of alkaline salts. Chyle is the material by means of which the blood is constantly renewed. It contains mostly some white and red blood-corpuscles, which leads to the idea that they might perhaps be formed in certain lymphatic glands through which the chyle has to pass. Before entering the blood, chyle is always mixed with a considerable quantity of lymph, which differs from chyle only by the absence of fat in emulsion. The lymph is transuded serum of the blood, which penetrates into the tissues and there performs its functions; it is then reabsorbed and carried back to the circulation by the lymphatic vessels. There are many derangements of the lymph and chyle which occur simultaneously with diseased glands in scrophula, and in tuberculosis, and it is probable that improper nutrition has the main share in the production of these derangements in a great number of young children.

Chyle.

Chemical constitution of the red blood-corpuscles.

The peculiar shape of the blood corpuscles appears to be partly dependent upon their chemical constitution. This latter is the product of their own powers and their interchange with those of the surrounding serum. They have a certain specific gravity, which is maintained or varied (in diseases, in different classes of

animals) by the quantity of certain chemical ingredients which are within them. Amongst these latter most notable by its red colour, and in the first place by its chemical constitution, is hematocrystalline.* This substance contains carbon, hydrogen, nitrogen, iron, sulphur, and oxygen, in the proportions indicated by the following formula: $C_{600}H_{960}N_{154}FeS_3O_{177}$. This leads to an atomic weight of 13,280, or if it be determined by the quantity of that most stable element, iron, an atomic weight of about 13,000. Persons who have not studied this branch of chemistry, and who perhaps in their handbook, do not read of atomic weights rising above 500, may wonder at the high atomic weight here assigned to hematocrystalline. But this body can now be obtained pure in quantity, and the analyses of crystals have always shown them to contain four tenths per cent. of iron. The crystals are mostly of the rhombohedric system, and appear in tetrahedra, octahedra, with and without prisms, or in prisms only. Their watery properly diluted solution, when examined in the spectroscope, shows two remarkable bands of absorption, and obscuration of the blue and violet end of the spectrum. As the blood of all vertebrate animals, when viewed

Hematocrystalline or cruorine.

Shape of crystals.

Spectrum of oxydised and reduced hematocrystalline.

When the blood crystals were first discovered, and their various shapes and properties found out (Funke, Kunde), it was believed and stated (Lehmann) that they consisted of a colourless substance (hematoglobuline) to which a coloured matter (hematine) adhered like a dye. As long as the researches on this subject moved on the microscopic field only, this idea was plausible; for many crystals are actually so thin as to appear colourless under the microscope. But the chemical and spectroscopical researches of the last few years have established the error of this conception and substituted the doctrine of the text.

within the living blood vessels, shows the same bands, we can assume that hematocrystalline is present in it as such, and not formed by the process of preparing the crystals. Hematocrystalline can be deprived of its oxygen, and then its spectrum changes to that of reduced hematocrystalline. Shaking with oxygen, restores the double-banded spectrum. The two bands therefore belong to arterial blood, the one band to venous blood (Stokes). Hematocrystalline contains as proximate constituents an albuminous body, which after separation remains amorphous and colourless, and hematine, which retains the colouring power, the spectral influence upon light, and the iron of the original substance, though all varied in kind, proportion and quantity. Hematine has an atomic weight of about 620, and from 7·5 to 8 per cent. of iron. It occurs together with hematocrystalline in the urine in cruenturesis (paroxysmal hematuria). It yields many remarkable products of decomposition. Its spectra in various solvents and in the reduced state, and the spectra of the new derivates, are very characteristic. The optical and chemical phenomena of hematocrystalline and hematine are applicable to medico-legal research, as affording the most certain diagnosis of blood upon the smallest quantities of material. A diminution of hematocrystalline in the body constitutes the disease termed "chlorosis" or "anæmia." It is either a specific ailment, or a symptom and consequence of chronic disorders, or acute, particularly tropical fevers.

Hematocrystalline splits up into albuminous body and hematine.

Besides hematocrystalline the blood corpuscles con-

Cerebric acid in blood-corpuscles.

tain a quantity of cerebric acid or of lecithine. This has been variously called myeline (Virchow), protagon (Liebreich), and other names, but it is probably the same body as that which can be extracted from the brain. We further have in blood corpuscles a certain quantity of what is called stroma. This is merely a name for a substance which is supposed to give them a shape. The stroma remains when the other bodies are extracted. It is a kind of chemical skeleton, and can be isolated (by freezing, for example) and investigated (Rollet). It seems to be very different from the albuminous matters combined in hematocrystalline, for it is soluble in ether, alcohol, and chloroform, when these agents are dissolved in serum. But it contains a small quantity of fibrino-plastic substance (Alex. Schmidt), namely paraglobuline, sometimes also termed globuline. This remains insoluble when the blood-corpuscles, previously separated from serum by solutions of salt, are deprived of hematocrystalline by water. The gelatinous paraglobuline, after shaking with water and ether, is soluble in solutions of salt, dilute alkalies, and water containing one per mille of hydrochloric acid. Brought in contact with fibrinogenous solutions it frequently produces fibrine.

Stroma.

Stroma contains paraglobuline.

The blood-corpuscles carry oxygen, which has great affinity for hematocrystalline, from the lungs to the most distant or hidden and internal parts of the body. They there yield it up to the tissues, principally the muscles or the oxydisable matters contained in their juices, and the tissues, thus oxydised, return the carbonic acid, water, urea, and other products into the

Function of the blood-corpuscles.

blood. The muscles may either oxydise immediately or store the oxygen in large quantities, particularly during sleep, by means of their red colouring matter, which is identical with hematocrystalline. Although the carbonic acid affects the colour and condition of the blood-corpuscles, nevertheless the latter are not carriers of the carbonic acid. This gas is in the serum.

Carbonic acid is in the serum.

It is there partly dissolved in the same manner as in soda or Seltzer water, but to a great extent it is combined with alkaline bases, particularly sodium. When the blood-corpuscles of the venous blood arrive in the lungs, they have undergone a change which consists in the partial oxydation of a small quantity of their hematocrystalline, and this is transformed into an acid

Hematic acid.

which I will call hematic acid. This blood-acid contains nitrogen. It is not similar to any of the acids we know. It is not volatile but fixed: it is evolved from the blood-corpuscles and passes into the serum at the very moment when the former arrive in the small breathing cells of the lungs. There the blood-acid combines with the sodium, and the carbonic acid is set free and is left to take its course, with water-vapour, through the lung tissue into the respiratory passages.*

* The excretion of carbonic acid from the lungs is an act of specific secretion, to which the presence of oxygen (and nitrogen) may be a supplementary advantage (as favouring diffusion), but is not essential. Hüter's observation of twin fœti, which were born living within the uninjured membranes and surrounded by the liquor amnii, and in that condition exhaled gas, is to me conclusive evidence of this, as also of my proposition that the act of first breathing is an act of secretion. This theory is fertile of explanations of many dark phenomena, such as the peculiar power of newborn children to sustain prolonged states of asphyxia.

In my research on cholera I have shown that in that disease the serum of the blood, being changed in its constitution in consequence of the alvine flux, refuses to perform the functions which it performed before. It exhausts water and other matters from the blood-corpuscles, and the latter thereupon cease to carry oxygen; oxydation does not take place any longer, hence the temperature falls, and the algid condition of cholera is produced. In yellow fever, and in a disease which occurs in England, paroxysmal cruenturesis, the hematocrystalline of a portion of the blood-corpuscles leaves them, and in the one disease decomposes and colours the skin yellow, in the other appears in the urine with a red or reddish-brown colour. A similar decomposition takes place in some cases of pyæmia, and in poisoning by arseniuretted hydrogen, and by the bite of venomous serpents. Several poisons, such as sulphuretted hydrogen, carbonic oxyde, hydrocyanic or prussic acid, kill or injure by decomposing the hematocrystalline, or combining with it, and keeping the oxygen out. Thus the study of many diseases requires an intimate knowledge of the constitution of the blood-corpuscles. The value of this we have only just begun to appreciate, and the chemical and optical methods of investigation applied with rigorous accuracy will bring us not only the explanation of normal phenomena at present remaining obscure, but also useful practical information on the nature of diseases, processes of poisoning, and their treatment by prevention or cure.

Blood change in cholera.

One of the most remarkable properties of the blood

Coagulation of the blood.

is its power of coagulating or setting shortly after it has left the body. Of this phenomenon many ingenious theories have been given, but none has yet satisfied the demands of exact chemical science. The latest and most plausible one is that of A. Schmidt, according to which the serum contains two substances, which have the power of combining with each other to form fibrine, the substance which produces the phenomenon of the curdling of the blood, as caseine produces that of the milk. One of these matters has been termed fibrinoplastic substance (paraglobuline) contained in serum and corpuscles, the other fibrinogen, contained also in serum and other fluids of the body. There is no doubt that substances answering to these descriptions can be isolated, and when brought together mostly form fibrine. But what is not yet proved is that they combine in atomic proportions. It is just possible that the paraglobuline only acts as a ferment like rennet, and transforms fluid fibrinogen into gelatinous, solid, or fibrous fibrine, by the power which in chemistry is termed contact action. But even should this theory be confirmed as at present shaped, it would fail to afford any answer to the obvious question as to the inhibitory power which prevents these bodies from reacting upon each other in the circulating blood. The cause of the coagulation being determined, would make room for the question as to the cause of the fluidity and non-combination of these bodies during life and circulation. This would no doubt be a progress, but not a solution. It is a fact that blood while in contact with the inner

Fibrinoplastic and fibrinogenous matter.

Circumstances under which blood keeps fluid.

surface of healthy blood-vessels in living bodies, even when prevented from flowing by double ligatures, does not coagulate. Even coagulated blood, when placed into the cavity of the excised heart of a turtle becomes fluid again. From these and similar facts the living walls of blood-vessels were said to be the agencies which kept the blood fluid (Brücke). But such a statement is only a circumscription, not an explanation of the fact, which therefore requires further study and investigation.

Fibrine contains albumen, physiolysis.

It must always be borne in mind that by the method of physiolysis, or putrefaction conducted upon certain principles and with certain precautions, fibrine yields albumen, and that consequently the atomic constitution of fibrine must be more complicated, and its atomic weight higher than that of albumen.

Chemical constitution of the serum.

The yellowish liquid which remains when the fibrine has been allowed to set in the blood, and to enclose and retain in its contracting meshes the blood-corpuscles, is termed the serum. It yet has a quantity of paraglobuline, of which only a small portion has been used up towards the formation of fibrine. It further contains sodium albumen (or caseine of serum, Panum), and a very small quantity of potassium albumen. The paraglobuline is precipitated by carbonic acid from serum diluted with 10 volumes of water, the albuminates dissolved in combination with the alkaline metals are set free by a little acetic acid. But the ingredient of serum which is present in the largest quantity (7·9 to 9·8 per cent.) is the albumen particular to the serum, or blood albumen, as distinguished from

Paraglobuline.

Sodium and potassium albumen.

Blood or serum albumen.

the albumen or white of egg. This is completely precipitated by boiling in the presence of a little acetic acid. If no acetic acid is present, sodium albumen, formed during the process of coagulation, remains in solution.

Neutral fats and soaps.

The serum contains neutral fats and soaps in solution or suspension, in the latter case being rendered milky or turbid thereby. In cases of disease which I have made known the serum also contains free fatty acids.

Free fatty acids in emulsion.

These acids are emulged with the phosphate of sodium of the serum, and after extraction by ether are again easily emulged by boiling with a solution of common sodium phosphate in water. A similar emulsion I have shown to occur in so-called chylous urine, and in certain effusions in the scrotum, termed variously milky hydrocele or lactocele or better lipo-rocele. The serum further contains cholesterine, kreatinine, urea, hippuric and lactic acid in small quantities, and at least one, perhaps two, yellow colouring matters are contained in it. In gout, uric acid as urate of sodium and calcium is found in it; in diabetes, sugar; in jaundice, biliary colouring matter, or cholophæine; and in leukocythæmia, formic acid, xanthine and other matters.

Mineral salts in serum.

Of inorganic salts the serum contains sodium chloride, sodium dicarbonate, and calcium phosphate, small quantities of magnesium phosphate, still smaller ones of potassium salts. The chemical operations of the serum (and blood on the whole) employ sodium salts mainly, while those of the muscles mainly choose salts of potassium. We shall see how the function of

the liver employs the sodium as the principal mineral base for its peculiar acids. But an explanation of this strict separation has yet to be found. We are more confused than enlightened by the discovery of the apparent paradox that fish, living in a medium abounding with sodium salts, should form a bile the mineral base of which is potassium.

Gases in serum.

The serum carries nearly the whole of the carbonic acid contained in the blood, particularly when it becomes venous. This is present in two forms, the one dissolved and removable by the vacuum, the other combined with soda as carbonate and dicarbonate, and dislodged only by acids and boiling. It is also possible that the sodium phosphate attracts a portion of carbonic acid and holds it in peculiar loose combination.

The physiology of the gases of the blood, of the oxygen, nitrogen, and carbonic acid contained in its modifications of arterial and venous character, has lately been improved by the invention of instruments and methods; but these do not yet satisfy all demands.

Quantity of blood in a man.

The total quantity of blood contained and circulating in a living man has for many years been greatly overestimated, owing to fallacious conclusions derived from the practice of bloodletting. The best methods available about ten years ago reduced the quantity to 7·7 per cent. of the weight of the body (Bischoff). But this has to be again reduced, as in these processes the myochrome was not deducted from the hematocrystalline, by the quantity of which the blood was estimated. Seven per cent. of the weight of the body will probably

be a more correct estimate of the quantity of blood than any other hitherto made.

Striated muscles. The striated muscles consist of particular contractile matter, disposed in layers within a fine membranous bag (sarkolemma) and connective tissue. The contractile matter is arranged in disks consisting of syntonine, which are laid close together, separated and surrounded, however, by a particular plasma. This can be isolated from muscles treated at the freezing point, taken out of the animal just killed, or from cold-blooded animals, such as frogs. The plasma is alkaline, and coagulates on standing like blood; by beating the coagulation is favoured. The coagulum is termed myosine; it is flaky, never fibrous, and more transparent than fibrine. Plasma dropped into warm water coagulates instantaneously, and deposits pure myosine. Like fibrine, myosine decomposes peroxyde of hydrogen. By solution in dilute acids it is transformed into syntonine. This is the name of the solid part of the flesh tissues, the particular fibrine of flesh of Liebig, which, insoluble in water, can be extracted from the insoluble part of meat by dilute acids in large quantities. The muscle-plasma, after coagulation of the myosine, leaves the muscle-serum. This becomes quickly acid, like all meat on keeping, and on neutralization deposits an albuminous substance. The fluid on acidification precipitates albumen which was in combination with potassium. This precipitation occurs in the meat naturally by formation of paralactic acid, which at first shares the potassium of the phosphate, and transforms it into acid phosphate. To this belongs the acid

Muscle-plasma.

Myosine.

Syntonine.

Muscle-serum.

First albuminous matter.

Potassium-albumen.

reaction of kept meat. Only when excess of paralactic acid in its free state is present does the albumen separate from the potassium-albuminate. Besides these two modifications the muscle-serum contains the ordinary serum albumen of blood. There is also contained in the serum of the red-coloured muscles a coloured albuminous matter, myochrome, identical with hemato-crystalline. In white muscles this matter seems to be absent. When flesh is extracted with water the albuminous matters contained in the solution are precipitated by boiling (albumen curdles at 65° C., hemato-crystalline only at about 90° C.), and a faintly yellow broth is obtained, which, when made of mutton, retains that name, but when made of beef is popularly termed beef-tea. This mysterious fluid, which is of great dietetic value to the healthy and the sick, contains a great number of remarkable ingredients—kreatine, $C_4H_9N_3O_2$; kreatinine, $C_4H_7N_3O$, the former a neutral body, the latter a powerful organic base, which also appears in the urinary excretion; uric acid, $C_5H_4N_4O_3$; xanthine, $C_5H_4N_4O_2$; hypoxanthine or sarkine, $C_5H_4N_4O$; guanine, $C_5H_5N_5O$; all of which appear in the urine of man or of the lower animals; taurine, the body obtainable from taurocholic bile-acid, and which may therefore, perhaps, be formed in the muscles, and carried to the liver, or be formed in the liver and carried to the muscles, or be formed in both in different ways; inosic acid, $C_5H_6N_2O_5$, and acids of analogous composition; further bodies free from nitrogen, as paralactic acid, $C_3H_6O_3$; formic, acetic, and butyric acids; glykogen, the same as that in the liver; dextrine; sugar, or

Serum-albumen.

Myochrome.

Beef-tea.

Ingredients of beef-tea.

grape-sugar, and a particular kind of sugar also found in the green shells of French beans, namely, inosite, $C_6H_{12}O_6 + H_2O$. The total of these bodies which are known amounts to two grammes from the extract of 1000 grammes of flesh; but the total amount of extract obtained is 12 grammes of organic substances. Consequently five sixths of the extract of meat are at present quite unknown to us. The extract of the meat of animals, oxen and sheep, has now become an article of wholesale manufacture and of trade. It contains about 18 per cent. of salts, of which nearly half the weight is potash; less than 18 per cent. of water, and of its dry residue 60 per cent. are soluble in alcohol of 80 per cent. strength. The economic advantages of this extract are the direct result of the purely scientific studies of Baron Liebig, and have made his name literally a household word. The residue of the meat from which the extract has been pressed contains all matters insoluble in water, syntonine, myosine, sarkolemmata, with nuclei, and fat. Some of this fat is visible under the microscope in the fibre, but some is invisible and dissolved, though not as soap. The residue contains phosphate of potash in insoluble combination with an organic matter, but no chlorides.

Extract of meat.

Residue of extracted meat.

The muscle is a machine for the transformation of chemical into mechanical force, and for the storing and exercise of that force. The exercise is partly constant and involuntary, partly intermittent and subject to the will. The loaded muscle is alkaline, full of disposable oxydisable matter, and of oxydant, namely, oxygen. The nerves of motor influence effect chemical contact

and immediate contraction of the various elements. The muscle becomes acid during work, and gives off large quantities of carbonic acid. The disintegration of its albuminous matter seems hardly to be increased immediately, but that of the carbonaceous substances is evident. Muscular exercise does not increase the quantity of excreted urea, but it augments that of the carbonic acid exhaled to perhaps tenfold the amount excreted in rest during equal times. The muscle participates in all febrile diseases of the body, and is frequently the seat of idiopathic processes. In typhoid and typhus fever it becomes disorganised in a high degree, losing structure, and assuming a yellow appearance. In fatty degeneration it loses its contractility, and shows fat in a free state amongst changed structure elements. In death from carbonic oxyde it has a red florid colour due to the combination of the poison with its hematocrystalline; in tetanus it is spasmodically contracted, changed, and frequently torn; in hydrophobia it is similarly injured and torn; in trichiniasis it is the specific seat of a parasite, the trichina spiralis, which does not live in any other tissue. The changes of the muscles in diseases have only just begun to be studied, and cannot fail to be found of the utmost importance. As the muscle during life takes up oxygen from the blood, besides nutriment of the most varied kind, and renders back to the venous blood carbonic acid, water, and a host of refuse matters, so does the dead muscle, its coagulated myosine notwithstanding, continue some time to breathe, take up oxygen and give out carbonic acid. At last its atoms take a new direction, and

Diseases in muscles.

Dead muscle.

Physio- and chemolysis of muscle.

physiolysis or putrefaction commences. The muscle yields the products common to the albuminous substances, tyrosine, leucine, volatile acids, and alkalies, &c. The same products are obtained by chemolysis; leucine was first discovered (by Braconnot) in the decomposed muscular tissue. By continuing the study of chemolysis we shall probably be able to learn more about the changes of the muscles in disease, as well as about the ingredients of flesh extracts which are at present unknown.

Involuntary muscles.

The smooth or organic involuntary muscular fibres contain some ingredients similar to those of the striated, but require to be better studied—a matter of great difficulty, as they cannot easily be isolated.

Ingredients of the brain and nerves.

The nerves and brain consist mainly of minutely thin long fibres, and of cells standing in connection with these fibres. These fibres are membranous tubes, filled with a peculiar nerve or brain marrow. When the nerves are taken out of the living body they change quickly, and the marrow of the tubes separates into two substances—a central one, the cylinder axis, and an outer or periaxial portion. This change is not yet quite explained. Possibly it may consist in a coagulation of the axial cylinder, which causes its contraction. The axial cylinder is, however, already in life of a different chemical composition from the periaxial tube, for it shows no effects in the polariscope, while the periaxial part exhibits dark or light crosses. The chemical constitution of these matters is perhaps the greatest problem of organic and physiological chemistry. A consideration of all researches since the time of

Vauquelin tends to show that brain matter is constituted similarly to the hematocrystalline of the blood; that it contains an albuminous matter to which is combined a substance containing nitrogen and phosphorus; that its molecule must therefore be very complicated and large, and that the matters hitherto extracted from the brain are at the most detached proximate nuclei, or single stones broken out of the mosaic picture which its molecule may be imagined to represent. The principal one of these detached nuclei is a body of which a small quantity can be extracted from brain matter by boiling alcohol, and which has been variously termed brain-wax, brain-fat; but by Frémy, who first obtained it pure and determined its composition, was found to be an acid, and termed cerebric acid. Lately Liebreich, having examined the same substance, gave it the name of protagon, but without any valid reason. This cerebric acid appears in crystalline needles, and gives remarkable reactions. By decomposition it yields fatty acids, glycero-phosphoric acid ($C_3H_9PO_6$) containing the whole of its phosphorus, neurine ($C_5H_{13}N$), choline ($C_5H_{15}NO_2$), and cerebrine ($C_{17}H_{33}NO_3$). This reaction makes it probable that it is proximately composed of cerebrine and lecythine ($C_{42}H_{84}NPO_9$), which latter yields the products last mentioned by the following reaction:

Brain-fat, cerebrine, cerebric acid, protagon.

Fatty acid, glycero-phosphoric acid, neurine.

$$\underbrace{C_{42}H_{84}NPO_9}_{\text{Lecythine.}} + 3H_2O =$$

$$\underbrace{C_3H_9PO_6}_{\text{Glycero-phosphoric acid.}} + \underbrace{C_5H_{15}NO_2}_{\text{Choline.}} + \underbrace{C_{18}H_{32}O_2}_{\text{Oleic acid.}} + \underbrace{C_{16}H_{22}O_2}_{\text{Margaric acid.}}$$

Much of it is contained in the brain in a state of such firm combination that even concentrated sulphuric acid does not easily set it free. There is further in the brain Cholesterine. cholesterine, the peculiar alcohol of which a quantity is constantly excreted with the bile. The albuminous substance of the brain has not yet been isolated completely; a small quantity can be extracted as potash- Caseine, albuminous matter. albumen (or caseine); another quantity is insoluble in water, and its presence can only be deduced from the curdling of brain-matter by boiling, and from the products of the chemolytic process, which, as I have found by special researches, yields all the products of chemolysis of albuminous matter, volatile acids and alkalies, leucine, tyrosine, and other substances. Of other Various ingredients. matters there are found in the brain substance lactic and a volatile acid, and inosite; uric acid, xanthine and hypoxanthine; kreatine. Sugar is sometimes found, but may be merely a product of transformation of cerebrine, and more rarely a substance which gives reactions similar to starch, and the study of which may perhaps throw some light on the peculiar disease termed amyloid degeneration. In diseases, leucine and a homologue of it appear sometimes. In others much urea collects in the brain and in the cerebro-spinal fluid, which fills the cavities of the brain and spinal marrow. The largest quantities of urea are met with in cholera, in which the cerebro-spinal fluid may contain as much as ordinary urine. In a case of softening of the brain Lehmann found glycero-phosphoric acid in the softened matter. The brain substance contains 25 per cent. of solids only, and 75 per cent.

of water. Its ash contains acid phosphates and a quantity of free phosphoric acid, a residue of the destroyed cerebric acid. Besides much potassium, some sodium, little magnesium and calcium, the ash always contains a notable quantity of iron. The ash of the grey matter of the brain is always alkaline, owing in part to the circumstance that it contains less cerebric acid than the white matter. When an ethereal extract of brain matter is mixed with water, and seen under the microscope, it swells and projects various peculiar forms of matter in various directions. The phenomenon is due to cerebric acid, which is dissolved in soap; it has given much and varied amusement to microscopists.

Ash.

Myeline.

The connective tissue forms tendons, fasciæ or envelopes of muscles and limbs, ligaments of joints and capsules, and binds all organs of the body together. It consists of fibres, of a cement uniting these to a tissue, of cells or so-called corpuscles, and is interspersed with elastic fibres. It is soaked with potassium-albumen. The cement is soluble in caustic lime and in baryta-water; it is reprecipitated by acetic acid, and then manifests itself as mucine, identical with that of the salivary glands and embryonic tissues, *e. g.*, the umbilical cord; the fibrillæ remain isolated, but mixed with the corpuscles or cells and their nuclei, and with elastic fibres. In acetic acid the fibrillæ are transformed into glutine or glue. The same transformation is effected by prolonged boiling in water. The elastic fibres resist this treatment. Both sorts of fibre by chemolysis yield tyrosine, leucine, volatile acids, and

Connective tissue.

Cement mucine.

Gelatine or glutine.

alkalies, but the gelatine is distinguished by yielding glykokoll, the lower homologue of leucine, while elastic fibres yield more leucine. Mucine yields sugar by chemolysis, and in this respect resembles chondrine, but it also yields much tyrosine, up to 7 per cent. These various data have given rise to the idea that mucine and glutine were products of a peculiar cleavage of albumen, but albumen has not yet been found to yield any sugar. The cells or corpuscles of the connective tissue consist of a semi-fluid matter which is termed the protoplasma, and of nuclei. In the protoplasma of the cells of the connective tissue pigment is frequently deposited (choroid, rete Malpighii of negro, bronzed skin disease, freckles, melanotic cancers), which is black or brown. Of this pigment very little is known.

Protoplasma.

Pigment.

Fatty tissue.

The fat tissue is made up of cells, in the interior of which fat is deposited, and of connective tissue. The fat cells are probably peculiar organs, but the cells of the connective tissue above described are probably able to deposit fat within their substance under certain circumstances. Some fat tissue remains permanently in the body, some may lose its fat during starvation or disease, and regain it afterwards. The fats in the human fat tissue are mostly those described in the paragraph on digestion, namely, tri-stearine, tri-palmitine, tri-oleine. The fatty tissue is subject to hypertrophy, or excessive infiltration; there are also peculiar colorations observed in several diseases, (*e. g.*, consumption or phthisis), which are due to an accumulation of luteine. Newly formed fat tissue is observed in the fatty

tumours or lipomata. Fat in tissue may originate in several ways; it may have been eaten with the food, and after absorption have only been carried to the cells; or it may be formed from sugar, dextrine, and glykogen; or lastly, it may owe its existence to the decomposition of albumen. Thus the cholic acid of bile might easily yield any of the fatty acids, and sugar the glycerine. But the proofs of any of these processes have not yet been furnished. Fatty tumour.

Cartilage contains peculiar cells and chondrinogen, or a substance which by boiling becomes chondrine. This matter gelatinises like gelatine, but is not soluble in acetic acid, on the contrary is precipitated by it. Boiled with hydrochloric acid, chondrine yields sugar. Cartilage.

The chemical composition of the cornea is similar to that of cartilage, particularly the hyaline variety. Cornea.

The fluids of the eye, particularly the vitreous body, contain much potassium chloride and little organic matter.

The cartilages are subject to a degeneration which terminates in ossification. Ossification.

The cartilage surfaces of joints are lubricated by a fluid which is termed synovia. It contains about 94 per cent. of water, 3·5 of albumen, 0·5 of mucine, and more than 1 per cent. of ash. Its origin is not yet satisfactorily explained. During diseased conditions of joints much synovia can be detected; the collection of excess of synovia in a joint constitutes hydrarthron. Synovia. Hydrarthron.

A matter similar to chondrinogen is hyaline, of which the cysts of old echinococci are composed. Boiled with dilute sulphuric acid it yields, like the chitine of Hyaline.

insects and articulate animals ($C_9H_{15}NO_6$) dextrose fermentescible sugar.

Bones. The bones consist of a peculiar combination of organic matter with mineral earthy phosphates, and contain as accessory matters marrow, blood-vessels, and cellular nuclei in the cavities called bone-corpuscles.

Osseine. The organic substratum is called osseine (a name which it would be better to apply to the entire bone tissue), the mineral matters are termed bone-earth. The osseine is obtained by extracting the bone-earth with dilute hydrochloric acid. It retains the structure of the bone substance, which is disposed in concentric layers round a tubular centre. Osseine dissolves on boiling in water like glutin, but differs from the latter in several particulars, although the product of the solution in hot water is true gelatine. Of osseine purified bones contain from 29·5 to 30·9 per cent., with which 68·1 to 69·4 per cent. of earthy salts are in combination. The regularity of these proportions has led to the assumption that bone is a chemical compound in definite atomic proportions of osseine and earths, and not merely a tissue in which earths are deposited. Some remarkable experiments show that gelatine and earthy phosphates have a peculiar attraction for each other. Thus a mixture of gelatine and bone-earth in hydrochloric acid, when neutralised by ammonia, deposits bone-earth with 20 per cent. of gelatine; *vice versâ* when tannic acid is added to such a solution tannate of gelatine with much bone-earth is precipitated. Bone-earth contains 9·1 per cent. of calcium

carbonate, 87·7 of calcium phosphate ($Ca_3P_2O_6$), 1·7 of magnesium phosphate ($Mg_3P_2O_6$), and 3 per cent. of calcium fluoride (CaF_2). The nutrition of the bones proceeds from their surface towards their cavities. Certain dyes, given with the food, penetrate into the bones and stain them. The idea formerly combined with this experiment, namely, that the bone was rapidly renewed from without and absorbed in the marrow cavity, is probably quite untenable. In several important diseases the bones are greatly affected, and become either brittle, or soft and bend. Such diseases are rhachitis, or rickets, common in children, the osteomalacia of pregnant women, and that particular form which attacks aged persons. In these diseases the bone-earth falls to about 30 per cent. of the dry bones, while the osseine rises to 60 and 80 per cent. The osseine at the same time changes its chemical character, by a curious tissue transformation, starting from the marrow cavities, and now no longer yields glutine. It seems that the agent by means of which in this no doubt complicated process the earths are removed is lactic acid, which under these circumstances, and not in health, is found in and about the bones. These diseases urgently call for chemical investigation.

Bones dyed with madder.

Rhachitis.

Osteomalacia.

Lactic acid in bones.

The teeth are bones, of which a part, the root, is constructed like ordinary bone; while the top or crown is formed upon a particular plan, round many minute tubes; this latter tissue is termed dentine. The outer hard covering, the cement, is an epithelial formation, containing only 4 per cent. of organic matter, 92 per

Teeth.

Dentine.

Cement.

cent. of earthy phosphates, and 4 per cent. calcium fluoride.

Pus. Pus is a product of disease or injury. It consists of a serum, in which many corpuscles, resembling the white corpuscles of the blood, are suspended. From Myosine in corpuscles. the corpuscles, separated by filtration, myosine (see muscles) is obtained. This substance is probably the instrument of the contractility of these bodies. The Paraglobuline, caseine, and serum albumen in serum. serum has fibrino-plastic properties, *i.e.*, contains paraglobuline, which can be precipitated by carbonic acid; on addition to the filtrate of acetic acid potassium albuminate or caseine is precipitated, and after the removal of this latter serum albumen is precipitated by heating. Chondrine and glutine. Mucine has never been found in pus. Chondrine and glutine have been found in the filtrate from the pus coagulated by boiling. They are probably connected with the corpuscles, like the glutine which is found in Leukocythæmia. the blood in leukocythæmia. Pus, probably in its corCerebric acid. Cholesterine. puscles, contains cerebric acid and cholesterine; the latter is deposited on standing. During standing and decomposition pus also deposits palmitic and stearic acid in crystals, oleic acid after addition of acetic acid. Fatty acids. Good or healthy pus from wounds contains no volatile Volatile fatty acids. acids, but decomposing pus yields volatile acids of the fatty series, formic, butyric, valerianic, in short—products of putrefaction. Pus from abscesses, from phosphorus disease, and ulcerating cancers, contains an acid, which as it gives a rose-pink reaction with Chlorrhodinic acid. chlorine water, has been termed chlorrhodinic acid. A similar substance can be obtained from decomposing pancreas extract and lymphatic glands. Pus may con-

tain leucine, tyrosine, xanthine, and uric acid; in the pus from jaundiced persons biliary acids and cholophæine are found; in that from diabetic patients sugar. The so-called blue pus derives that colour from a kind of vibrio, which yields its pigment to chloroform, from which it is obtained in crystals, and termed pyocyanine. The solid matters contained in pus amount to from 10 to 15 per cent.; its ash is similar to that of blood, contains 72 per cent. of sodium-chloride, and more potassium-salt than blood-serum. A knowledge of the composition and products of decomposition of various kinds of pus from abscesses, ulcers, wounds, &c., is of the utmost importance for the study and treatment of reactive fever after wounds and operations, of various forms of blood disease termed septichæmia, and pyæmia. In all these affections pus, or products of its decomposition, are absorbed or enter in a more grossly mechanical manner into the lymph and blood, set up an acute patholytical process which leads to violent attacks of shivering, fevers, sweating, diarrhœa; then, in the case of septichæmia and pyæmia to secondary deposits of pus or other fluids in various organs and cavities, particularly the lungs and liver, and ultimately, and in nearly all cases, to death. Against this fatal disease a particular kind of treatment of wounds, the so-called antiseptic treatment, has lately been devised, and is now under the consideration of surgeons.

Blue pus.

Pyocyanine.

Pyæmia.

The function of the spleen is not well known, but seems connected with the elaboration of certain constituents of the blood and certain processes of digestion. The organ contains much blood, and a

Spleen.

separation of its tissue and juice from its blood has not yet been effected. The fresh spleen is alkaline, but soon becomes acid. The watery extract contains hematocrystalline and the other ingredients of blood, besides a peculiar albuminous matter which on combustion leaves phosphoric acid and iron oxyde. It encloses some cholesterine. After removal of all albuminous matters there are in the extract of fixed acids the lactic and succinic, of urinary products hypoxanthine, xanthine, and uric acid, of volatile fatty acids formic, acetic, butyric, of amido-acids leucine; of alcohols there is inosite in considerable quantity. These ingredients show that chemical processes of various kinds must be carried on actively in the spleen. They are, however, not indispensable to life, as animals from which the spleen has been removed by operation continue to live without any perceptible disturbance. In leukocythæmia the spleen is frequently very large, and weighs up to nine and ten pounds. If it be found small in that disease the lymphatic glands are certainly enlarged. The spleen is sometimes subject to (here) miscalled amyloid degeneration. Although in this state it gives many reactions of albumen it is indigestible in artificial gastric juice; it gives no sugar by treatment with sulphuric acid, and is little prone to change by artificial or natural influences.

Peculiar matter.

Ingredients of water-extract.

Waxy degeneration of spleen.

Thymus gland.

The thymus gland is a mysterious organ situated in the chest in front of the lungs. It becomes of less importance to the adult than it probably is to the fœtus in the womb. It contains albumen, collagene, elastic tissue, a little fat, leucine, xanthine, hypoxanthine,

succinic and lactic acid, and perhaps also volatile fatty acids and sugar. In the progress of the involution of the thymus the amount of sodium contained in it is nearly doubled.

Thyroid.

The thyroid contains nearly the same chemical constituents as the thymus. It would be very confusing that the constituents of glands of the most varied connection and situation are identical, were we not reminded that the defined ingredients are perhaps only one fourth or one sixth of the whole of the ingredients, and that specific differences may therefore be discovered upon the ingredients which at present are undefined. The thyroid is said to contain mucine, particularly when in the state termed colloid degeneration; it then also contains cholesterine. When containing brown fluids, in the disease termed struma, a sediment of blood-corpuscles is mostly present, which, however, contain only decomposed hematocrystalline in the form of hematine.

Suprarenal capsules.

The renculi yield many curious coloured reactions. Their alcoholic or ethereal extracts become yellow and red when exposed to the air, and show a green fluorescence due to renculine; their watery extracts are coloured red by iodine, and blackish blue by iron-chloride. They contain leucine, but the presence of other particular biliary matters which has been alleged is at present not proved. In certain chronic diseases (Addison's disease, or bronzed skin), in which the skin is more or less copper or brown-coloured, the suprarenal capsules are specifically diseased.

The ovaries are composed of the stroma, the Graafian

Ovaries. follicles and the corpora lutea. The latter contain fluid and coagulable serum, particularly while they possess an internal cavity. In their substance is deposited in granules luteine, a yellow matter soluble in alcohol, ether, and chloroform, and distinguished by three absorption-bands which its solutions show in the blue and violet part of the spectrum. A similar yellow matter is contained in the yelk of eggs. The yellow colour of corpora lutea is not due to hematine as has hitherto been generally assumed.

In some forms of ovarian cyst a yellow albuminous fluid is contained, which shows the spectrum of luteine without any preparation; in this morbid fluid the luteine (cysto-luteine) is therefore contained in solution, while in the normal corpora lutea it is contained in granules (ovario-luteine).

Salivary glands. The salivary glands contain mucine and leucine, xanthine and hypoxanthine.

Pancreas. The pancreas contains much leucine, a homologue of it, xanthine, hypoxanthine, and guanine. Also some of the ferments mentioned in the paragraph on digestion can be extracted from its pulp as well as from its juice.

Liver. The liver consists of cells, which are the main seats of its specific function, and of blood-vessels and bile ducts, interspersed with lymph vessels and nerves. The blood can be washed out by water. The cells are made up of a protoplasma, which may contain as visible ingredients fat in large and small granules, cholophæine in small red granules, nuclei, with one or two corpuscles, and as invisible ingredients demonstrable by experiment only, a coagulable matter which sets soon after death,

albumen, mucine, and glykogen. Sugar and biliary acids are also always obtained from the extract of the liver, although the ducts may have been carefully washed out; and as cholophæine is contained in the cells, we may assume that the bile acids are also contained and made in the cells. The fresh liver is always alkaline, but on standing it becomes acid. The glykogen is mostly or entirely transformed into sugar. The liver-extract made by boiling water mostly contains lactic acid and volatile fatty acids, inosite, hypoxanthine, xanthine, and uric acid, and leucine. The latter body occurs in livers which are quite fresh, in very small quantity, but increases by decomposition, for which the tissue of the liver or its cells possess particular aptitude. Fat is extracted from most livers, in large quantities from diseased livers. Tyrosine occurs in diseased livers only, and is not easily obtained even from thoroughly putrid livers. In the ash of the liver phosphoric acid and potassium predominate, which is the more to be noted as the bile acids in man are mainly combined with sodium. From fresh livers of young persons and animals hydrogen is sometimes evolved on immersion in warm water. The degeneration of the liver, which is sometimes wrongly called amyloid, or otherwise the "Speckleber" of the Germans, and "waxy degeneration" of the English, has not any resemblance to the amyloid degeneration of the spinal marrow: for iodine and sulphuric acid, or iodine alone, produce in it only a red or reddish-brown mahogany-like coloration, its material being evidently of an albuminous kind: and on extraction by alcohol

Bacony liver.

and ether, I have found in it considerable quantities of cholesterine, fat, and paralbumen, but neither bile nor sugar. Having during many years, and in many cases, observed considerable quantities of leucine in the liver, and having frequently experienced great difficulty in isolating this substance, and separating it from others, I subjected to a special study the compounds of leucine with metals, and discovered a new copper compound which renders leucine entirely insoluble in water and neutral fluids. For the extraction of hypoxanthine and xanthine I have also devised special processes.

Leucine, special method for isolation.

Kidneys.

The kidneys contain much blood, collagene fibres, and some fat; to water extraction they yield chlorrhodinic acid, uric acid, and bodies resembling hypoxanthine and xanthine, but urea is scarcely obtainable from them. Cystine and inosite have on some rare occasions been met with. The chemical changes of the kidneys in diseases have not yet been sufficiently examined.

The urine.

The urine is the secretion of the kidneys. It is the lixiviated refuse from the chemical processes of the body. It contains a yellow colouring matter, urochrome, which by chemolysis yields various remarkable products of decomposition. The first is uromelanine, $C_{36}H_{43}N_7O_{10}$, a most interesting substance, with an atomic weight of 733, being one of the highest at present established in organic chemistry. Accompanying uromelanine there is a small quantity of a matter which by treatment with sulphuric acid yields the reaction and spectrum of cruentine, termed paramelanine. The next product is uropittine, not as yet sufficiently studied.

Uromelanine

Uropittine.

Then there is omicholine, empirical formula $C_{22}H_{38}NO_5$, an organic base with fluorescent properties and a peculiar spectrum, and omicholic acid, $C_{15}H_{22}NO_4$, also fluorescent, but slightly differing in its spectrum and composition from omicholine; further, acetic and formic acid. From these products it is evident that urochrome must possess a very high atomic weight; it may, perhaps, be a derivate of hematocrystalline; uromelanine no doubt represents the nucleus of hematine. The ingredient which occurs in urine in the largest quantity is urea, CH_4N_2O, of which a man secretes about 30 grammes in twenty-four hours. Then there is kreatinine $C_4H_7N_3O$, the same as that which occurs in the muscles. It is partly changed into kreatine, $C_4H_9N_3O_2$, by taking up an atom of water during the process of preparation. From kreatinine is derived sarkosine, which is isomeric, or perhaps identical with alanine $C_3H_7NO_2$. There is further contained in urine uric acid, $C_5H_4N_4O_3$, which may form calculi and cause much trouble, and a series of bodies being less oxydised than uric acid, namely, guanine, $C_5H_5N_5O$, hypoxanthine, $C_5H_4N_4O$, and xanthine, $C_5H_4N_4O_2$. Further, there is an acid which from having been first discovered in horses' urine is termed hippuric, $C_9H_9NO_3$, remarkable by its consisting of a combination of glykokoll $C_2H_5NO_2$, and benzoic acid, $C_7H_6O_2$, less an atom of water, H_2O, and by its being formed in considerable quantity in the human body when benzoic acid is being taken, or greengages are consumed. The extractives are at least three in number; of these I have fully identified kryptophanic acid which if considered

Omicholine.

Omicholic acid.

Urea.

Kreatinine and kreatine.

Sarkosine.

Uric acid group.

Hippuric acid.

as dibasic has the formula $C_5H_9NO_5$, but which must perhaps be considered as tetrabasic, and then has the formula $C_{10}H_{18}N_2O_{10}$; in that case its metallic salts will have the general formula $C_{10}H_{14}M_4N_2O_{10}$. Another extractive acid is paraphanic acid, $C_{11}H_{18}N_2O_6$, dibasic.

Allantoine. Under some circumstances allantoine appears, $C_4H_6NO_3$, which can also be produced from uric acid artificially, along with other remarkable products. Of inorganic

Salts. salts the phosphates of lime and magnesia, and of potash, and potassium chloride, are present in considerable quantity, but most prevalent is the sodium-chloride or common salt. Of some matters minute traces appear

Sugar, alcohol. in the urine, thus of sugar and of alcohol, after these bodies have been taken by deglutition. In diseases

Pathological ingredients. there may appear blood, albumen, fibrine (paraglobuline and fibrino-plastic matter), fatty acids and fats, large quantities of sugar, as in diabetes, leucine, tyrosine, abnormal colourless matters yielding by acids urocyanine and urorubine, as in cholera; oxalic acid and oxalate of lime, as in a particular disease which frequently ends in the formation of calculi. Much as the urine has been studied its chemistry is by no means accomplished, and on the causes of the most troublesome diseases showing themselves by symptoms in the urine, gout, uric acid calculus, oxalic diathesis, diabetes, chylous urine, our knowledge is as yet very incomplete.

Under all circumstances, however, the analysis of the urine is an indispensable aid to clinical diagnosis, and furnishes most valuable positive and negative information on acute as well as chronic diseases.

Sweat. The secretion of the skin, discharged in large quan-

tities after exertion or under the influence of a heated atmosphere, contains much surface epithelium. Of chemical ingredients there are observed lactic and sudoric acid, the latter peculiar to sweat and not found anywhere else, and urea. There is much sodium-chloride, little or no phosphate. Sometimes volatile fatty acids, *e.g.* valerianic, are found in small quantity, but it is possible that they are formed after the sweat has been secreted. Lactic and sudoric acid.

In respiration oxygen of the air inhaled together with its nitrogen is absorbed by the blood, and in exchange carbonic acid and water are given out. The expired air therefore contains less oxygen than the inspired, and a quantity of carbonic acid instead. But the whole of the oxygen inhaled does not return as carbonic acid and water; a portion is otherwise combined and leaves the body in the urinary products of oxydation, particularly urea. A healthy strong man exhales in twenty-four hours upwards of 400 litres of carbonic acid, and inhales upwards of 500 litres of oxygen. The expired air during rest contains about four volumes per cent. of carbonic acid. During activity the expiration of carbonic acid becomes much more rapid, and in extreme cases may for a short time rise to tenfold its normal quantity in the same time. Activity or muscular work on the other hand hardly increases the quantity of urea excreted by a man. Breath.

The breath in diseases may contain carburetted hydrogen (of which a trace is also excreted in health) and volatile matters at present unknown, ammonia being perhaps amongst them. These investigations have only just become possible by the invention of an apparatus

which permits entire persons to be observed for days in glass chambers, and their excretions to be accurately analysed and determined. By means of this method it has now been found that man during sleep stores up a quantity of oxygen in his body, particularly his muscles, which is therefore ready for the production of force the moment it may be wanted. This explains the phenomena of activity and rest much better than they could hitherto be defined.

Fæces. The fæces contain all insoluble residues of the food, some decomposed and altered bile acid, changed cholophæine, myochrome, cholesterine changed into excretine, some peculiar fatty and odoriferous matters, besides a little phosphate of magnesium and calcium. They vary with the diet, being dark, semifluid, and of small bulk after meat diet, but of large bulk, paler and more solid, after bread diet. Boiled with sulphuric acid they yield a matter which has a peculiar spectrum.

The fæces of children at the breast contain cholesterine, and besides caseine and undefined matter, a yellow matter soluble in alcohol, intestino-luteine. This possesses one absorption-band in its spectrum, at the beginning of blue.

The anomalies of fæces are still less known than their normal composition. The cholera evacuations were fully described in my report on cholera. Their spectrum may be compared with stercorine on the one, and acid cruentine on the other hand.

Iron preparations taken into the stomach colour the fæces black, mercurial ones green. This latter fact gave rise to a fallacy now exploded, namely, that mer-

cury was a cholagogue and increased the excretion of bile. Bile (not decomposed) has never yet been found in any fæces. Most remarkable and suggestive is the discovery by Liebig of alloxan in the mucous evacuations from a case of intestinal catarrh. Alloxan is a product of decomposition by oxydants of uric acid, and precedes the formation of urea. Possibly, therefore, functions of chemolysis are allotted to the intestine which at present we place into other organs, or know not where to localise. Gout might find an explanation in the failure of this chemolytic action, for uric acid once in the blood seems as far out of the reach of oxygen as sugar in the blood is in diabetes. Imperfect digestion further causes the production of gases, of which carbonic acid constitutes the main bulk, but is mixed with combustible marsh-gas, CH_4, and some hydrothion H_2S. This development in diseases rises to painful height, and in typhus, *e.g.*, produces sometimes an almost suffocating tympanites or meteorismus.

Alloxan in mucous evacuations.

Gases in intestines.

ANALYTICAL GUIDE.

Acetic acid, $C_2H_4O_2$.—1. *Extraction from animal substances.* Extract the substance with boiling water; neutralise by soda if acid, and if dilute, evaporate to a reasonable bulk. Distil with dilute sulphuric or phosphoric acid; neutralise the distillate with soda, and evaporate to the consistence of an extract. Treat this like the extract of urine, now to be described.

2. *Extraction from urine.* Evaporate fresh urine to an extract, and decant from crystals of sodium chloride and other deposited matters. Mix with a sufficient quantity of sulphuric acid to decompose nearly all the urea, and distil the mixture from a retort. Neutralise the distillate with sodium carbonate, and evaporate to a low bulk. Add excess of somewhat dilute sulphuric acid; allow benzoic acid to crystallise and filter. Neutralise the filtrate again with soda and evaporate to a low bulk; and if you desire to prove the presence of and extract *formic acid*, proceed as stated under that head. Then evaporate the soda salt to dryness, and heat in a retort with excess of concentrated sulphuric acid. The formiate will be destroyed, yielding carbonic

oxyde and carbonic anhydride, while the acetic acid will distil over unchanged and highly concentrated, at a temperature not exceeding 120° C.

3. Place this concentrated distillate in a tube in a mixture of ice and water. It will begin to form white crystals below 16° C., and ultimately solidify in a white crystalline mass.

4. Allow to thaw, and pour off the portion first liquefied, which is somewhat watery.

5. Place the crystals in a very small retort, provided with a thermometer in its tubule, and with a condenser attached to its neck, and heat. The acetic acid will distil over completely at 120° C., giving an inflammable vapour.

6. Unite the decanted and distilled acid, and dilute with water. Boil with excess of pure baryum carbonate, filter, and evaporate to crystallisation. The crystals, monohydrate of baryum acetate, $C_4H_6BaO_4 + H_2O$, contain 6·59 per cent. of water of crystallisation, to be expelled at 100° C., and 50·18 per cent. of Ba, to be determined as sulphate, by heating in platinum crucible with sulphuric acid.

7. Heat some dry baryum acetate; it will fuse, and give off vapours of acetone.

8. Dissolve the baryum acetate in water, heat, and decompose exactly with sodium carbonate. Filter from baryum carbonate, and examine the solution of sodium acetate as follows.

9. Add a few drops of ferric chloride: a dark red colour will be produced. Boil; a brown-red precipitate will fall, leaving the liquid colourless. To another

portion of the red solution add hydrochloric acid. It will turn light yellow.

10. Add silver nitrate solution. If the sodium acetate solution is concentrated, a white precipitate of silver acetate will ensue. If both solutions are dilute no change will be observed even on boiling.

11. Evaporate the sodium acetate solution to dryness. Heat a portion with alcohol and sulphuric acid. Acetic ether will be formed and recognised by its peculiar and agreeable odour.

Albumen, or white of egg.—1. Break a fresh hen's egg, separate off the yelk and chalazae; dilute the white with three or four times its bulk of water, filter, and use the clear liquid for the following experiments.

2. Place a portion in a test-tube, insert a thermometer, and warm gently in a water bath; at 66° C. the solution will become opalescent, and about 80° C. the albumen will coagulate, and be precipitated in white flakes, which do not dissolve on boiling.

3. Add a little nitric acid; the albumen will be precipitated white.

4. Add an excess of strong alcohol; the albumen is precipitated.

5. Add a solution of corrosive sublimate, and observe that an insoluble compound of the salt and albumen is precipitated. Upon this reaction is based the use of white of egg as an antidote in cases of poisoning by corrosive sublimate.

6. Acidify a portion of the albumen solution with acetic acid, and observe that no precipitate is produced.

But on boiling the whole of the albumen is precipitated. After filtration and drying weigh the precipitate. This is a good method for estimating the quantity of albumen present in any colourless liquid.

7. Add a watery solution of creasote, or of crystallised carbolic acid, or of tannic acid, to an albuminous solution. The albumen is precipitated.

8. Add a solution of salt to the albumen solution, and then phosphoric, tartaric, oxalic, or lactic acid. A precipitate of albumen will ensue.

9. Expose some undiluted albumen in a thin layer on a white porcelain plate to the air. It will dry into a pale yellowish, translucent, fissured mass, which is slowly but entirely soluble in water.

10. Chemolyse albumen by the following process :— Boil the white of several eggs with excess of dilute sulphuric acid for three hours. Then treat the solution with milk of lime until alkaline. Distil the mixture from a tin bottle, and observe in the distillate ammonia, a compound ammonia, and a volatile sulphur compound, which yields sulphur and sulphuretted hydrogen on addition of an acid. Extract with water, and filter the residue in the bottle. Treat the solution with a slight excess of dilute oxalic acid; remove the excess of the latter and some sulphuric acid by a little lead acetate, and the excess of the latter by sulphuretted hydrogen. Filter warm, and evaporate to slight crystallisation. All tyrosine with little leucine will crystallise out. Further evaporation will yield more leucine. The liquor contains several other products, which are distinguished by a powerful green fluorescence. Examine

this in the cone of sunlight produced by a lens. Examine tyrosine and leucine as directed under those articles.

11. Add to coagulated albumen some concentrated hydrochloric acid, and heat or allow to stand for some time. The albumen will dissolve and form a blue or violet solution, which, before the spectroscope, shows an absorption band in yellow and green.

12. To coagulated albumen add concentrated nitric acid; it will slowly dissolve, forming a yellow solution. To this add a small quantity of mercurous nitrite and boil, when a crimson precipitate will form.

Alcohol, C_2H_6O.—1. In order to obtain alcohol from organic tissues, or fluids which may contain it, heat, coagulate, and distil them or their cold prepared watery extracts from a copper or tin retort. To urine add some tannic acid before distillation. The distillate obtained is to be made alkaline with caustic potash, and again distilled. Observe, if desirable, volatile acids in residue in retort. The distillate is now acidified with sulphuric acid, to fix volatile alkali, and again distilled. This third distillate contains all the alcohol, but no volatile acids or alkalies.

2. Produce a test solution by dissolving one part of dichromate of potassium in three hundred parts of sulphuric acid.

3. Mix a portion of the distillate with twice its volume of concentrated sulphuric acid. Pour a small quantity of this mixture into a quantity of test solution, and perceive that where the one fluid touches the

other there is a deep green, and then a lighter green, colour produced. One fourth to one tenth of a grain of alcohol in half an ounce of water will yet be indicated by this test. Prove this by experiments with pure alcohol.

4. By the foregoing process you can prove the presence of alcohol in from two to six ounces of urine secreted a few hours after the drinking of spirituous liquors.

5. Quantitative determination of small quantities of alcohol by transformation into acetic acid. Enclose the distillate with a certain quantity of the dichromate and sulphuric acid mixture in a strong flask, stop it air-tight with a caoutchouc stopper, and tie it down well by means of wire. Heat this flask for two hours in a water-bath to between 80° and 90° C., never to the boiling-point; then attach the flask to a condenser, and distil the newly-formed acetic acid out of it. Determine the acidity of the distillate by volumetric analysis, with a standard solution of caustic soda.

6. In case larger quantities of alcohol are contained in the matters to be examined—*e. g.* vomited matter, contents of stomach or intestines, blood, brain, muscles, or urine in quantity—then the distillate can be condensed by successive distillations, in which one half of the liquid is driven over every time, and the liquid in the retort is each time mixed with common salt. Ultimately the distillate will smell of alcohol, and burn when exposed to flame. It can then be made anhydrous by boiling with and distilling from quicklime or anhydrous copper sulphate. Its specific gravity at

0° C. should be 0·8095; at 14° C., 0·7982. It should boil at 78° C. under a pressure of 760 millimetres of the barometer.

Allantoine, $C_4H_6NO_3$.—1. Glassy, tasteless prisms. Obtain from the allantoic liquid of cows by evaporating to one fourth of its bulk. The crystals of allantoine deposited on cooling redissolve in hot water, and boil with a little good animal charcoal.

2. From the urine of calves. Obtain the urine by tying of bladder during killing process. Evaporate it till syrupy, and allow to stand for several days; dilute with water, collect the deposit and wash it with a little water. Boil the crystalline residue with water and animal charcoal and filter hot. The allantoine will be deposited in crystals on cooling.

3. Prepare allantoine from uric acid as follows:— Take 20 grm. of uric acid, stir up in 300 to 400 c. c. of water, add a small quantity of acetic acid, gradually introduce 100 grm. of lead peroxyde, and expose to sunlight for some time. Boil, filter, and evaporate the filtrate until on cooling the allantoine crystallises out.

4. Observe that it is slightly more soluble in alcohol than in water.

5. Boil with baryum hydrate, and notice evolution of ammonia.

6. Corrosive sublimate will produce no precipitate in its solution, but mercuric nitrate precipitates it in the cold even from dilute solutions.

Alloxan. — $C_4H_2N_2O_4$. — 1. Add to strong nitric acid, contained in a beaker placed in cold water, an

equal weight of uric acid in small portions, stirring or shaking the mixture constantly. Allow to stand and crystallise, filter through a funnel plugged with asbestos, wash the crystals with water at 0° C. and dry between filtering paper.

2. Extract organic matters, such as intestinal contents or evacuations in disease with water, and subject to dialysis on parchment paper. Evaporate the dialysate at a gentle heat and ultimately let dry spontaneously. If alloxan be present it will be deposited in crystalline rings which assume a red colour when exposed to the air for some time.

3. Dissolve alloxan in water or alcohol and add nitric acid, the alloxan will be reprecipitated.

4. Observe its astringent taste and acid reaction by litmus. Rub a solution of it on a part of the skin, and observe that it produces a peculiar and disagreeable odour and stains the skin pink, crimson, or purple, after some time.

5. Boil some alloxan with dilute nitric acid in a test-tube fitted with a cork and bent tube, the latter dipping into a little lime or baryta water. Carbonic anhydride will be evolved, causing a milky turbidity in the lime or baryta water. Nitrate of urea remains in the acid solution, which will give a dense white precipitate with solution of mercuric nitrate.

6. Add to a solution of alloxan some solution of ferrous sulphate, and a drop of potash; observe the formation of a deep blue solution and precipitate. This solution after filtration shows no specific absorptions before the spectroscope.

7. To a solution of alloxan add some baryta water and solution of baryum nitrate, and observe the formation of a pink-red solution. This, before the spectroscope, shows a specific absorption band covering orange, yellow, green, and blue, and allowing red and a part of blue with violet to pass.

8. Drop into a concentrated solution of lead acetate heated to boiling a solution of alloxan, and observe the formation of a white precipitate of mesoxalate of lead, and the liberation of acetic acid. From the filtrate urea can be obtained by removal of the lead and evaporation of the acetic acid.

Amyloid substance.—1. Search the brain, spinal marrow, ependyma ventriculorum, ganglion Gasseri, or optic nerve in disease, particularly so called chronic atrophy or locomotor ataxia, by hardening the substance to be examined in strong alcohol, then making the thinnest possible sections with a razor and placing them under the microscope.

2. Apply tincture of iodine dissolved in iodide of potassium, and observe that no blue starch granules appear.

3. Wash away the iodine solution by water and apply dilute sulphuric acid, let stand for some time and apply some iodine solution. The amyloid, if present, will now appear in the form of minute blue granules closely resembling the iodised starch corpuscles of wheat.

4. Compare the reaction of amyloid matter with that of vegetable lignine.

Animal quinoidine.—1. Treat the part to be exa-

mined, either directly or after previous drying in a water-oven, with very dilute sulphuric acid, heating the mixture on the water-bath. Repeat this extraction, mix the extracts, filter after cooling, neutralise with caustic soda and repeatedly shake up with ether equal in bulk to that of the extract. Distil off the ether, take up the residue in dilute sulphuric acid, filter, and observe if fluid be clear.

2. If not clear repeat neutralisation with soda and extraction with ether, and resolution in dilute sulphuric acid.

3. Treat the solution for fluorescence by exposing it to a cone of sunlight, or of the spark of the Rühmkorff coil, condensed by a quartz-lens. Observe that the colour produced is bluish-green when the solution is concentrated, but blue when dilute.

4. Compare this fluorescence with that of a very weak solution of sulphate of quinine in water, and observe its close resemblance to it.

Benzoic acid, $C_7 H_6 O_2$.—1. Obtain benzoic acid by the process described under Acetic acid, 2.

2. Obtain benzoic acid by boiling hippuric acid with excess of concentrated hydrochloric acid. It will crystallise on cooling. Filter and wash with cold water. Dry in the air without using heat.

3. Purify the acid by sublimation from a porcelain capsule covered with a diaphragm of filtering paper and a conical hood of paper.

4. Heat a small quantity of the acid in a glass tube, and observe that it fuses, then is volatilised and again

deposited on the cool parts of the glass. The aromatic smelling vapour causes much coughing when inhaled in small quantity.

5. Heat a small portion with concentrated sulphuric acid and observe that no blackening ensues.

6. Neutralise its hot watery solution with baryum carbonate, and evaporate solution of baryum benzoate to crystallisation.

7. Add to the solution of this or any other benzoate some neutral solution of ferric chloride, and observe the precipitation of a buff-coloured precipitate of almost insoluble ferric benzoate. For diagnosis of this precipitate from succinate, see Succinic acid, 4.

8. To a solution of a benzoate or of benzoic acid add some cupric acetate and warm; a blue precipitate of rather insoluble basic benzoate of copper will be formed.

Bile.—Qualitative and systematic analysis. 1. Collect the bile from the gall-bladders of dead persons or animals, or from biliary fistulæ of the latter produced by art. Observe its colour, formed or crystalline ingredients if any, and its reaction, which should in health be neutral or feebly alkaline.

2. Add to a filtered portion a little acid, and, if necessary, filter again. Then heat gently to boiling, and if any precipitate ensues albumen is present.

3. Mix the bile with five or six volumes of absolute alcohol, and let stand; filter from the precipitated mucus and cholesterine (and albumen if such was abnormally present). Evaporate the solution to dryness, and use the residue as purified bile.

4. Purified bile dissolved in absolute alcohol, and mixed with an equal bulk of ether, forms an immediate deposit. Place this mixture in freezing air, or into a freezing mixture, and after twenty-four hours observe that crystals are formed and the liquid is clear. These crystals are a mixture of glykocholate and tauro-cholate of sodium. Evaporate the ether and observe the crystals of cholesterine and fats.

5. Add to a watery solution of purified bile neutral lead acetate as long as a precipitate is produced. The white plaster-like deposit is glykocholate of lead.

6. Add to the filtrate, from the glykocholate, a solution of basic lead acetate as long as a precipitate is produced. The latter consists of taurocholate of lead.

7. Filter the liquid and treat with hydrothion; filter again, evaporate to dryness and burn the residue to a white ash. Observe that it consists principally of sodium carbonate, little potassium carbonate, some chlorides, and earthy salts in small quantity.

8. Boil a quantity of purified bile dissolved in water with excess of caustic baryta for several hours. Separate solution from insoluble salt and crystals which form on cooling, and remove excess of baryta by carbonic acid. Add to the concentrated solution platinic chloride, and observe and examine crystalline precipitate of choline-platinum chloride.

9. Remove from this solution the excess of platinum by hydrothion, evaporate to small bulk and add much absolute alcohol. Let stand for twenty-four hours, then isolate crystals, recrystallise from hot water, and study the pure taurine.

10. From the alcoholic solution from which taurine has been deposited, evaporate off the alcohol and dissolve residue in a little water. Remove hydrochloric acid by a little silver oxyde, the latter by hydrothion, and crystallise filtrate. Separate the crystals of glykokoll from the crystals of inorganic salts and examine them.

11. Add to crude or purified bile dissolved in water, a little acetic or hydrochloric acid, and extract the mixture with chloroform. Distil off the chloroform and extract the residue with boiling alcohol. Fatty acids and cholesterine will dissolve, and bilirubine will remain undissolved, as a red powder.

12. Add to crude bile a very small quantity of acetic or hydrochloric acid, and shake with animal charcoal until colourless. Wash the charcoal with water until the water comes away pure. Then extract the charcoal with much boiling alcohol. Bilifuscine will go into solution and form a brown liquid.

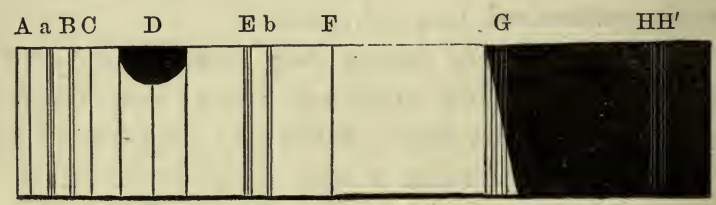
13. Evaporate a weighed quantity of bile to dryness and determine dry residue. It will be found to exceed five per cent., and approach ten per cent. of the weight of the bile.

14. Burn a weighed quantity of dry bile with nitre in a platinum dish, and in the residue determine sulphuric acid by baryta in the usual manner. From the quantity of sulphur found calculate the quantity of taurocholic acid present in the original bile. One part of sulphur indicates 16·28 parts of taurocholic acid.

15. To some bile diluted with water and spread on a

white porcelain dish add a few drops of red nitric acid. A precipitate will ensue around every drop, and then a red, blue, green, and yellow coloration will proceed in rings from each drop as a centre, due to the reaction of the colouring matters with nitrous and nitric acid.

16. To a few drops of bile placed in a porcelain dish add a drop of a solution of cane-sugar, and then concentrated sulphuric acid drop by drop with agitation. After a little time the mixture will assume a purple-red colour, and show the following spectrum:



Spectrum of Pettenkofer's test.

The colour of the solution will be destroyed by water and alcohol.

17. In diseased conditions of the bile, in fatal cases of cholera, the liquid contained in the gall-bladder contains no bile-acids.

Observe that in such cases the purple reaction just described cannot be obtained even with the concentrated alcoholic extract.

18. Boil purified bile dissolved in water with excess of hydrochloric acid in a flask for several hours, until a clear reddish fluid is obtained and a dark pitchy resin is deposited. The resin is choloidic acid and

dyslysine, which contains, however, some undecomposed glykocholic and taurocholic acid. Decant and filter the acid liquid, and evaporate to dryness repeatedly to expel all free acid. Then shake up residue in spirit and let stand. Taurine will deposit in crystals; hydrochlorate of glykokoll and chloride of sodium will remain in solution. Separate the latter by crystallisation or by the process described under 10.

19. Filter fresh bile through a cloth, or let stand for twenty-four hours, and decant the clear portion. Place in a stoppered bottle in a cool place or cellar for several weeks or months. After that time filter red liquid from deposit. The liquid contains a new acid, which gives precipitates with the chlorides of calcium and baryum, such as are not obtainable from fresh bile. The deposit after washing and pressing should be treated with boiling alcohol. Cholic acid will dissolve and will deposit in crystals on cooling. Bilirubine, and phosphates of lime, and magnesia with ammonia in crystals, coloured greenish by impurity, will remain. Extract the bilirubine by chloroform, and purify the earthy salts by calcination, or analyse them by the ordinary processes.

Bilifuscine (probably $C_9H_{11}NO_3$). — 1. Powder a brown human gallstone and extract cholesterine with boiling ether. Treat the powder with water and a little hydrochloric acid, and wash it to neutrality. Then extract again with boiling ether to remove fatty acids, and boil the powder with absolute alcohol. Bilifuscine will form a brown solution and remain after

evaporation of the alcohol as a black, shiny, brittle mass, or as a dark brown powder.

2. Dissolve a small quantity in dilute potash lye, and reprecipitate by hydrochloric acid brown flakes.

3. Dissolve in dilute ammonia, and add chloride of calcium or baryum, when bilifuscate of calcium or baryum will fall down in brown flakes.

4. Observe that bilifuscine is not soluble in chloroform, and when exposed to the air in alkaline solution does not yield biliverdine.

5. Spread an alkaline solution on a white dish, and add a drop of red nitric acid. Red, blue, green, violet, and yellow coloured rings will be successively produced.

Bilirubine, synonym *Cholophœine*, $C_9H_9NO_2$.—1. Extract some powdered oxgallstone successively with water, alcohol, dilute hydrochloric acid, boiling alcohol, and ether; then boil the dry powder with dry chloroform, and exhaust with this agent. Distil the chloroform from the red solutions, but not quite to dryness. To the residue add several volumes of absolute alcohol and let stand twenty-four hours. There will be deposited a brilliant red powder mixed with steel-blue or brown crystals. Both the powder and the crystals are pure bilirubine, and can be separated by levigation with much absolute alcohol. After washing with alcohol and ether, until the washings are purely yellow and not green, the product is pure. Dry under the air-pump.

2. Human gallstones, by the foregoing treatment,

will, after extraction of the bilifuscine, also yield bilirubine, but in very small quantity only.

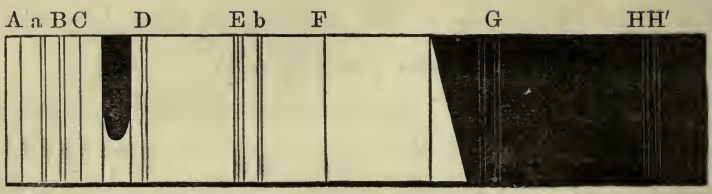
3. Human bile or ox bile will by putrefaction deposit bilirubine. See Bile, 19.

4. Saturate some very dilute aqueous solution of ammonia with bilirubine in excess, and filter quickly. In this solution nitrate of silver produces a precipitate of neutral monohydrated cholophæinate of silver $C_9H_{10}AgNO_3$. In the same solution chlorides of baryum and calcium produce red precipitates of the half-acid salts, sesqui-cholophæinates, which contain three atoms of cholophæine, two atoms of water, and one (didynamic) atom of metal, yielding the formula $C_{27}H_{29}M^{II}N_3O_8$.

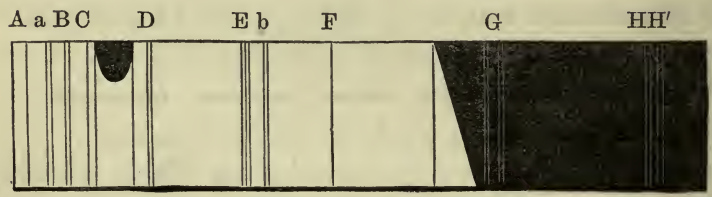
5. Dissolve some cholophæine in excess of ammonia. To this solution add chloride of calcium or baryum, and observe the formation of dark red precipitates of the neutral salts of the formula $C_{18}H_{20}MN_2O_6$.

6. Dissolve some bilirubine in caustic or carbonated alkali, and expose to the air for some days, frequently shaking the solution with air. Observe that the red solution becomes purely green. Then precipitate by hydrochloric acid, wash the green flakes by decantation, and dissolve in absolute alcohol. The latter on evaporation will leave pure *biliverdine* $C_8H_9NO_2$.

7. Add to an ammoniacal solution of cholophæine concentrated nitric acid drop by drop until a blue precipitate is formed. Isolate quickly by filtration, and after washing dissolve in alcohol. This blue solution of *cholocyanin* has the following spectrum:

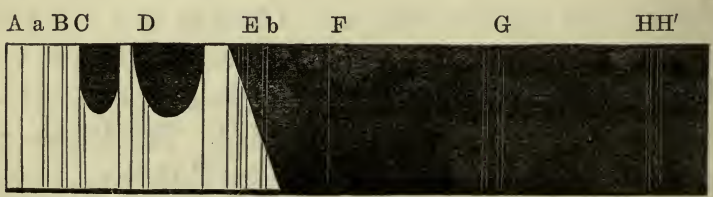


Spectrum of Cholocyanine.



Spectrum of Cholocyanine dissolved in water.

8. Treat some dry cholophæine with fuming or much concentrated sulphuric acid, and triturate in a mortar; let the green solution attract water from the air, and then add more water. Several green products insoluble in water will be formed. One of these, insoluble in alcohol, is *cholothalline*, $C_9H_{11}NO_3$. Another, soluble in alcohol, has the following spectrum:



Spectrum of soluble Cholothalline.

9. Expose some dry bilirubine to the vapours of bromine, and observe that it immediately becomes violet-brown. Exposed to the air this will deliquesce and form a blue solution of hydriodate of dibromo-

bilirubine. Dried at 100° the acid will be expelled, and pure dibromo-bilirubine, $C_9H_7Br_2NO_2$, will remain as a black-blue powder. It dissolves in water with the aid of any acid, and forms a splendid blue solution. It also dissolves in alcohol and a little in ether, but these solutions are discoloured after some time. It dissolves in caustic alkali with a red colour, and acids produce a red precipitate. These tests under 9 can be performed with a quantity of less than a grain.

Blood.—1. Take a quantity of blood directly from a blood-vessel of an animal or of man, and stir it briskly with a rod for ten minutes. Filter through a cloth and wash the *fibrine* with water until colourless.

2. If it is desired to determine *the quantity of the fibrine* weigh the collected blood in a stoppered bottle containing a chain of glass beads, shake for ten minutes, add water, let stand and deposit, decant the fluid, wash with water by decantation, ultimately with water containing a little chloride of sodium, collect, dry, and weigh the fibrine.

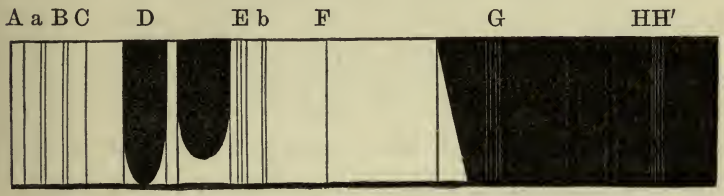
3. Mix one volume of saturated chloride of sodium solution with from nine to ten volumes of distilled water. To this solution add one volume of blood, beaten and filtered through calico, and stir. Let the mixture stand at a very low temperature in ice and water. When the corpuscles are deposited decant the supernatant liquid, and stir the deposit again with the same quantity of salt water as at first. Repeat this washing operation a third and fourth time, when the *blood-corpuscles* will be free from serum.

4. Shake the blood-corpuscles thus freed from serum with ether and water, and observe that the red *hematocrystalline* dissolves in the water. Filter this solution without delay, and expose to a low temperature. If the blood came from dogs, rats, guinea pigs, or squirrels, it will crystallise at once, but if it came from birds will crystallise only after the addition of one quarter of the volume of the solution of alcohol of 80% strength, and exposure to a cold of from —5° to —10° C. Separate the crystals by filtration, wash with alcohol of 20% strength by volume, press them between filtering paper, redissolve in a minimum of water, filter again, mix with one quarter volume of alcohol of 80%, and expose again to the low temperature. The crystals of *hematocrystalline* will re-form in a purer state than before. If the blood employed in this process be human or veterinary, no crystals, but only an amorphous deposit of hematocrystalline, will be obtained.

5. Burn a quantity of these crystals in a platinum crucible and observe that they leave a quantity of *red iron oxyde* corresponding to 0·43% of metallic iron in the crystals. Determine the other elements and observe that all analyses lead to the formula $C_{600}\,H_{960}\,N_{154}\,Fe\,S_3\,O_{177}$, giving an atomic weight of 13280.

6. Place a concentrated solution of hematocrystalline in a test-tube before the slit of the spectroscope, and observe that it excludes all light but the red. Then dilute the solution with water, and observe that green and blue light passes, while in yellow and the beginning of green a dark space remains. On further

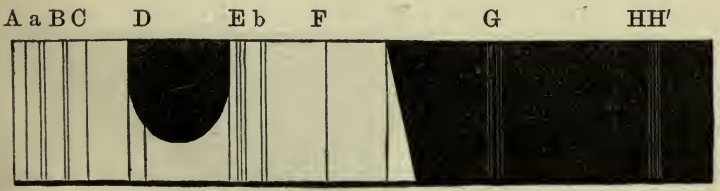
dilution the latter is seen to consist of *two absorption bands*, one, situated towards the red, close upon the D line, is narrower, darker, and better defined, while



Spectrum of Blood.

the second one situated towards the green on the side of the E line, which is towards D, is wider and paler. Dilute the solution gradually and observe how the bands become paler. Study these phenomena upon all kinds of red blood and red muscular tissue.

7. Treat a solution of blood which exhibits the two absorption bands with hydrogen or arseniuretted hydrogen gas, or with a solution of ferrous sulphate containing tartaric acid and excess of ammonia, the air being in each case excluded from contact with the mixture, and observe that the colour of the solution alters to purple, and that before the spectroscope only *one broad band* is seen in the place of the former two.



Spectrum of reduced Hematocrystalline.

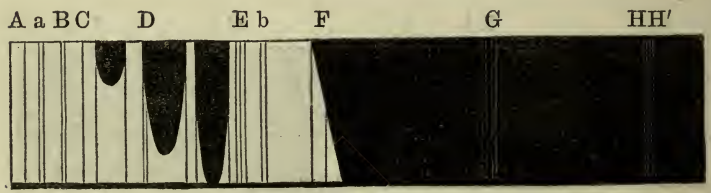
8. Shake up the solution prepared according to the

previous paragraph with air, and observe that the one band disappears and the two bands reappear. The one band is peculiar to *reduced or venous*, the two bands are characteristic of *oxydised or arterial* hematocrystalline.

9. Treat some diluted hematocrystalline solution or blood with carbonic oxyde, and notice that the colour becomes more bluish than either arterial or venous blood, and that the spectrum is that of arterial blood, but that the bands are moved a little more towards the violet end. This *combination of carbonic oxyde with hematocrystalline* cannot be dissolved by any reducing agent, and is the cause of death in cases of poisoning by charcoal vapours.

10. Treat some diluted blood with nitrous oxyde gas and observe that the absorption bands become a little paler. This is due to the expulsion of oxygen from and the *combination of nitrous oxyde with the hematocrystalline*.

11. Treat a solution of blood with hydrothion or ammonium sulphide, and observe that the spectrum becomes changed, three bands as in the following engraving making their appearance.



Spectrum of Blood treated with hydrothion.

12. Treat some hematocrystalline with acetic or

sulphuric acid and alcohol, and observe the formation of a solution of hematine. (See Hematine.)

13. Boil blood-corpuscles isolated by the salt-water process under 3 with alcohol, and extract *cholesterine* and *lecithine*. From the insoluble residue, decomposed hematocrystalline, extract hematine by acid and alcohol, and study albuminous residue like coagulated albumen.

14. Evaporate the filtered ether solution obtained under 4, and observe cholesterine and lecithine in the residue. Identify cholesterine by its crystalline form, and lecithine by its leaving on combustion *free phosphoric acid.*

15. Treat blood-corpuscles isolated by the salt-water process under 3 with water without stirring much. The hematocrystalline will dissolve and a gelatinous matter, the *fibrino-plastic substance*, will remain undissolved. Add some ether and alcohol to curdle the matter more completely, filter and wash. The matter is soluble in solution of sodium chloride and in water containing one tenth per cent. of hydrochloric acid.

16. Collect a quantity of blood and, without stirring it, let stand in a quiet cool place for twenty-four hours. Remove the clear *serum* with a pipette or syphon, and study as follows :

17. Shake a portion with ether, and observe *fats or fatty acids* in the residue of the ether solution.

18. Mix a measured or weighed portion of serum with several times its bulk of water, acidify with acetic acid, and boil. The *albumen of serum* will be precipitated.

Determine its quantity by washing, extracting with boiling alcohol, and weighing the dried precipitate. It will probably amount to between 7·9 and 9·8 % of the serum.

19. Evaporate the filtrate from the albumen precipitate to a low bulk, and test for tyrosine, leucine, kreatinine, urea, glukose, and extractive acids.

20. Burn the residue and examine the white *ash*, which should amount to about 0·7 to 0·8 % of the serum. Study the prevalence of sodium over potassium salts; of chlorides over sulphates; of alkalies over earths; prove the presence of phosphoric acid.

21. Dry a weighed quantity of serum, and determine total quantity of dry residue.

22. Dilute clear serum with ten volumes of water, and pass a current of carbonic anhydride through the solution. Let stand and allow the *fibrino-plastic substance* (also termed *paraglobuline*) to deposit.

23. Dilute the solution, from which fibrino-plastic matter has been deposited, by an addition of ten volumes of water, and neutralise most cautiously with very dilute acetic acid. A milky turbidity and a subsequent adhesive deposit are formed and constitute *fibrinogenous matter*.

24. Add to hydrocele fluid, or to the fluid of pericardial, pleural or peritoneal exudations, a small quantity of fibrino-plastic substance, and observe that they immediately deposit fibrine.

25. Shake some defibrinated blood in a stoppered bottle with much air or oxygen, and observe that it

assumes a bright red colour, and evolves carbonic anhydride. Identify the latter by passing it through solution of baryta or lime.

26. Shake some oxydised red blood with carbonic oxyde, and observe that the latter gas is absorbed while oxygen is expelled. Shake some oxydised blood with carbonic acid, and observe that some oxygen is expelled and carbonic acid absorbed, while the blood assumes a dark red-purple colour.

27. Expose blood enclosed in a piece of sheep's gut to chlorine gas, and observe that the red colour of blood passes into green.

28. Expose blood so enclosed to a mixture of ammonia and sulphide of ammonium gas, and observe its decomposition and dark discoloration.

Brain.—1. Free the brain-tissue from blood by injecting water into the blood-vessels while in the cranium, or after removal, and separate the membranes. Ascertain the specific gravity of the white matter to be about 1041, that of the gray matter about 1034.

2. Dry gray matter in a water bath and ascertain loss of *water* to be from 75 to 88 %, and the remaining *solids* to amount to 25 to 14 %; whereas *white* matter will lose less than 75 % of water, generally about 71 per cent., and leave 25 to 29 % of solids.

3. Extract *cerebric acid* with hot ether, as directed under *Cerebric acid*.

4. Extract *cerebrine* and *protagon* with boiling alcohol, as directed under those bodies.

5. From the cold ether extract containing cholesterine and lecithine which is obtained in the preparation of cerebric acid, lecithine is isolated by the process given under that substance, any great excess of the platinic or cadmium chloride being avoided. The filtrate from the precipitated lecithine compound is boiled with excess of plumbic hydrate and filtered. On cooling a copious crystalline deposit will form, from which pure *cholesterine* may be obtained by recrystallisation from boiling alcohol.

6. Rub brain-matter in a mortar with water containing enough sodium chloride to prevent cerebrine and cerebric acid from forming an emulsion; filter the mixture. The clear filtrate will contain *potassium-albumen* or *casein*, which may be precipitated by sulphuric acid not in excess. From the filtrate addition of a few drops of acetic acid and boiling will throw down albumen if present.

7. Rub in a mortar to a thin milk with excess of baryta water, heat till just coagulated, filter, remove the main bulk of the baryta from the filtrate by a current of carbonic acid, and the last traces by exact precipitation with sulphuric acid and evaporate to a low bulk. Divide this extract into two portions.

8. One portion of the extract distil with dilute sulphuric acid to obtain the *volatile acids*, *acetic* and *formic*. Examine them as directed under those bodies.

9. The other portion of the extract heat with ether

slightly acidified with sulphuric acid. Mix the ethereal solution with water, distil off the ether, remove any sulphuric acid by cautious addition of baryta water, evaporate the filtrate and test for *lactic acid* (*q. v*) by the zinc salt, and for *urea*.

10. The part insoluble in ether must be diluted with water, freed from sulphuric acid by addition of just sufficient baryta water, the filtrate evaporated to a low bulk and exhausted with boiling absolute alcohol. Leucine, kreatine, and urea will dissolve, and some leucine may crystallise out on cooling. From the alcoholic solution expel the alcohol by distillation till the aqueous residue forms not too thick a syrup, adding a little water, if necessary, and set aside to crystallise. *Kreatine* and *leucine* will separate while urea will remain in the mother liquor, and may be isolated by nitric or oxalic acid. (See Urea).

11. The kreatine and leucine may sometimes be mechanically separated (the former being in crystals, the latter in opaque granules) and afterwards be purified by crystallisation from alcohol. If not, dissolve the mixed deposit in hot water, and boil with zinc chloride. On cooling and standing, kreatinine, zinc chloride, and kreatine will separate in granules (see those bodies). From the liquid after precipitation of the zinc with ammonium carbonate and boiling, and evaporation of the filtrate, leucine (and homologues) may be isolated by the process given under leucine (*q. v.*).

12. The portion insoluble in boiling alcohol must be exhausted with boiling water. Some *uric acid* may

remain undissolved. To the boiling solution add neutral acetate as long as any precipitate is produced. Filter.

13. The precipitate may contain *lead urate* as well as lead, salts of inosic and similar acids. Suspend in a moderate quantity of water and decompose by sulphuretted hydrogen. The lead sulphide will retain most of the uric acid, which may be extracted by boiling with water, while the filtrate will contain the *inosic acid* and *allied bodies*, if present.

14. The filtrate from the precipitate by neutral lead acetate must be precipitated by basic lead acetate and cupric acetate. The solution filtered from these precipitates may contain *tyrosine*, which must be identified by evaporating, dissolving the crystals or deposit in a little hydrochloric acid, precipitating by sodium acetate, and applying the mercury nitrate and nitrite test. (See Tyrosine).

15. The precipitate may contain xanthine, hypoxanthine, and inosite. Suspend the precipitate in a rather large quantity of water, decompose by hydrothion, filter, extract the lead sulphide with boiling water and evaporate the filtrate and extract together to dryness. From the residue extract the *inosite* by cold water, and purify by crystallisation. The remaining *xanthine* and *hypoxanthine* may be separated and purified by the processes described under those bodies.

16. Burn a portion of dried mixed brain-matter carefully in a platinum dish (best in a muffle) and analyse the ash, noticing that there is always a quantity of

free phosphoric acid present in it. Observe that it contains 1·74 % of ash, of nearly the following composition :

Acid potassium phosphate	55·24
„ sodium „	22·93
„ iron „	1·23
„ calcium „	1·62
„ magnesium „	3·40
Free phosphoric acid	9·15
Sodium chloride	4·74
Potassium sulphate	1·64
Silica	0·42
	100·37

17. Burn another portion very gradually, adding hot saturated baryta water to the charring and charred matters from time to time, in order to neutralise the free phosphoric acid formed by the destruction of lecithine, and preserve the whole of the chlorides and sulphates intact. Prove by comparison that the acid phosphates found by process 16 are mainly formed by the phosphoric acid from the lecithine, expelling chlorine and sulphuric acid from the glowing ash.

18. Burn some dry white brain-matter, and notice that it leaves about 1·72 % of ash containing *free phosphoric acid* and a large amount of acid phosphates.

19. Burn some dry gray brain-matter, and notice that the quantity of its ash is only 1·16 %, that it is *alkaline*, and contains a much smaller quantity of phosphates than the ash from white matter.

20. Treat a minute fibre of brain-substance or a bundle of nerve-fibres under the microscope with perosmic acid, and notice a blue reaction.

21. Search for *amyloid matter* by the process described under that body.

22. In some diseases, such as softening of the brain, search for *free glycero-phosphoric acid.* This will pass into the liquids obtained by sodium chloride, process 6, and by baryta water, process 7. The sodium chloride solution, freed from caseine and albumen, as above, must be filtered, neutralised with calcium carbonate, evaporated to a small compass, and precipitated boiling with a sufficient volume of alcohol. Dissolve the precipitate in as little cold water as possible, and boil; *calcium glycero-phosphate* will separate, and must be again dissolved and thrown down by boiling, finally washed with a very little hot water, then with alcohol, dried at 100° C., and analysed. It should contain 60·47 % of Ca.

23. To obtain glycero-phosphoric acid from the baryta water extract, process 7, take the mother liquor of kreatine and leucine, remove urea by oxalic acid, and subject the residual fluid to the treatment described in the previous paragraph.

24. The albuminous matters which remain insoluble in the course of any of the processes of extraction above described, will yield by chemolysis (*e. g.*, by boiling with dilute sulphuric acid) leucine, tyrosine, and the other usual decomposition products of albumen.

Butyric acid.—1. Produce butyric acid from sugar, as directed under *lactic acid*, but allow the mixture to stand five or six weeks. The calcium lactate is

gradually converted into butyrate. When no more gas is evolved dilute with water, precipitate with sodium carbonate, filter, evaporate down and decompose with rather dilute sulphuric acid. Separate off the oily stratum of butyric acid, distil it with a little sulphuric acid, digest with fused calcium chloride and again distil, rejecting the first portions.

2. Distil the vomited matters or intestinal discharges of cholera patients (rice water) with sulphuric acid, neutralise the distillate with soda, evaporate down, decompose with sulphuric acid, and distil again. Saturate the hot distillate with hydrated oxyde of copper, and let stand, when *cupric butyrate* will crystallise, while cupric acetate will remain in solution.

3. Butyric acid boils at 157° C.; it has an unpleasant rancid smell; it is insoluble in water and alcohol.

4. To lead acetate solution add a little butyric acid dissolved in water; an oily precipitate of lead butyrate will appear.

5. To a solution of potassium or sodium butyrate add cupric sulphate; a green precipitate of cupric butyrate will be formed soluble in hot water, and depositing in crystals on cooling.

6. Heat a butyrate with alcohol and sulphuric acid. Butyric ether, having a characteristic odour of pine-apples, will be evolved.

Calculi, biliary. Systematic analysis.—1. Heat a portion of the calculus on platinum foil; it burns with a clear or sooty flame, and is almost entirely consumed.

Boil a portion of the calculus with alcohol; it is dissolved, and on cooling deposits crystals of cholesterine. These features characterise the pellucid or *pure cholesterine calculus.*

2. The calculus is coloured blackish brown or green, and contains a coloured nucleus. It burns like the former at first, but is with difficulty consumed, leaving a notable amount of ash. Extract cholesterine by ether, and test the coloured residue with dilute hydrochloric acid. From the washed residue extract *bilifuscine* with alcohol, and *bilirubine* with chloroform. The insoluble residue on combustion and the hydrochloric extract on evaporation will yield earths and their phosphates, particularly calcium and magnesium. This bearing characterises *mixed calculi with prevalence of cholesterine.*

3. The calculus is dark red or brown, rough, fissured, without fatty feel, and easily broken. A portion heated on platinum foil evolves a disagreeable odour of burnt feathers and leaves an ash. Treat a portion with water and hydrochloric acid and chloroform at the same time. The chloroform will become red from dissolved bilirubine. Evaporate the chloroform solution on a white plate, and add a drop of red nitric acid to the yellow residue, and observe the play of colours peculiar to the colouring matter of bile. These reactions characterise the *calculi with prevalence of cholochrome*, which occur rarely in man, but are the only calculi hitherto observed in cows and oxen.

4. The calculi are small and black or dark green, and insoluble in most solvents, except boiling nitric

acid. In this solution water produces an orange-coloured deposit. Neither cholesterine, nor bilifuscine, nor bilirubine, can be extracted from them, but they contain besides the coloured peculiar ingredient much earthy salt. They constitute the variety known as *calculi with prevalence of modified cholochrome*, and are found in old and decrepit persons.

5. Heat a portion of the calculus, it evolves the odour of burnt feathers, and on combustion leaves a slight ash. Treat a portion with alcohol and a little sulphuric acid, and observe its solution in the alcohol. Treat a portion of the calculus, or of the alcoholic extract with sulphuric acid and sugar as directed under cholic acid, a violet colour is produced. These reactions characterise calculi *with prevalence of biliary acids.*

6. Heat a portion of the calculus and it will evolve an odour of burning fat. Treat a portion with acetic acid and boiling alcohol and it will dissolve, and on cooling deposit fatty acids. Determine the melting-point of these, and test their reaction with a boiling solution of phosphate of sodium. The calculus may contain biliary pigments: *Calculi with prevalence of fatty acids.*

7. Heat the calculus or a portion and observe that it is merely coloured, hardly contains organic matter, and that its ash almost retains the shape of the matter before heating. Add hydrochloric acid to the ash, or to the original calculus, and it will dissolve with effervescence. Over-saturate the solution with ammonia and no, or scarcely any, precipitate will ensue. Such

are the reactions of *calculi with prevalence of carbonate of lime.*

Calculi, intestinal.— From horses fed upon bran. Saw the calculus in two halves, and observe nucleus. Examine saw-meal or a chip of the hard crystalline matter. It loses water and ammonia by heating to redness, and leaves a residue easily soluble in hydrochloric acid, and reprecipitated, on standing in crystals, by excess of ammonia. The calculus, therefore, consists mainly of *ammonio-phosphate of magnesium*, with which more or less *phosphate of calcium* is mixed. It hardly contains any inorganic matter.

Calculi, prostatic.—Minute concretions, from the size of mustard or hemp seeds to that of barley-corns. Dissolve powder in acetic acid, and observe evolution of carbonic acid gas. Add to solution excess of ammonia, and observe that solution remains clear; absence of phosphates of earths; if solution forms deposit phosphates of earths are present. If necessary filter, and add oxalate of ammonium; a copious precipitate of calcium oxalate will ensue. The calculi, therefore, consist principally or entirely of *carbonate of calcium.*

Calculi, urinary.—Systematic analysis. Powder the calculus. Heat a small portion of the powder to redness on some platinum foil and observe whether any residue is left which will not burn off.

A. In case it leaves a fixed residue, take a small

portion of the original calculus, dissolve in concentrated nitric acid, evaporate to dryness on a water bath in a white porcelain evaporating dish; dip a glass rod into the strongest ammonia, and bring it near the residue in the dish, and observe whether a pink colour is produced or not.

I. A pink colour is produced, proving that the calculus contains *uric acid;* observe whether a portion of the calculus melts on being heated.

a. It melts—

1. And communicates a strong yellow colour to the flame of a spirit lamp or Bunsen burner; *Sodium urate.*
2. And communicates a violet colour to the flame, giving the potassium spectrum; *Potassium urate.*

b. It does not melt; dissolve the residue left after ignition in a little dilute hydrochloric acid, add ammonia till alkaline, and then ammonium carbonate solution.

1. A white precipitate falls; *Calcium urate.*
2. No precipitate; add some hydric sodic phosphate solution; a white crystalline precipitate falls; *Magnesium urate.*

II. No pink colour is produced. Observe whether a portion of the calculus melts on being heated strongly.

a. It melts (fusible calculus). Treat the residue with acetic acid: it dissolves; add to the

solution ammonia in excess; a white crystalline precipitate falls; *Ammonio magnesium phosphate.* In case the melted residue is insoluble in acetic acid, treat with hydrochloric acid; it dissolves. Add to the solution ammonia; a white precipitate indicates *Calcium phosphate.*

b. It does not melt; moisten the residue with water, and test its reaction with litmus paper; it is not alkaline. Treat with hydrochloric acid, it dissolves without effervescence. Add to the solution ammonia in excess, white precipitate; *Calcium phosphate.* Treat the calculus with acetic acid; it does not dissolve. Treat the residue after heating with acetic acid, it dissolves with effervescence; *Calcium oxalate.* Treat the original calculus with acetic acid, it dissolves with effervescence; *Calcium carbonate.*

B. The calculus on being heated does not leave a fixed residue. Treat a portion of the calculus with nitric acid, evaporate and expose to ammonia vapour as before.

I. A pink colour is developed.

a. Mix a portion of the powdered calculus with a little lime, and moisten with a little water; ammonia is evolved and a red litmus paper suspended over the mass is turned blue; *Ammonium urate.*

b. No ammonia; *Uric acid.*

II. No pink colour is developed.

a. But the nitric acid solution turns yellow as it is evaporated, and leaves a residue insoluble in potassium carbonate; *Xanthine.*

b. The nitric acid solution turns dark brown and leaves a residue soluble in ammonia; *Cystine.*

Caseine.—1. Add to skimmed milk a little hydrochloric acid, wash the curd with water, then with water acidified with hydrochloric acid, and finally with plain water. Dissolve the jelly (caseine hydrochlorate) in a large quantity of water, filter, add ammonium carbonate cautiously, wash the precipitate (free caseine), exhaust with alcohol and ether, and wash again with water.

2. Dissolve the moist caseine thus obtained in dilute hydrochloric acid, filter. The liquid will rotate polarised light to the left, and will possess the properties of a solution of albumen.

3. To a weak solution of caustic potash add moist caseine as long as dissolved. The alkali will be neutralized. Filter and use for the following experiments:

4. Add an acid—the caseine will be precipitated, but will redissolve in excess of the acid.

5. Boil the solution. It will not coagulate, but an insoluble pellicle will form on the surface.

6. Add solution of a calcium salt, or of magnesium sulphate. A precipitate will appear *on boiling.*

7. Add solution of lead acetate, cupric sulphate,

alum, mercuric chloride, or tannic acid. A precipitate will appear *in the cold.*

8. Dissolve moist caseine in acetic acid. Add solution of potassium ferrocyanide or chromate; the caseine will be precipitated.

9. Dissolve moist caseine by warming in concentrated hydrochloric acid. The solution will have a fine violet colour.

10. Expose moist caseine to the air. It will putrefy, and yield similar products to fibrine (see fibrine).

11. Boil caseine with caustic potash. Ammonia is evolved. To the liquid add lead acetate. A black precipitate will prove the presence of potassium sulphide.

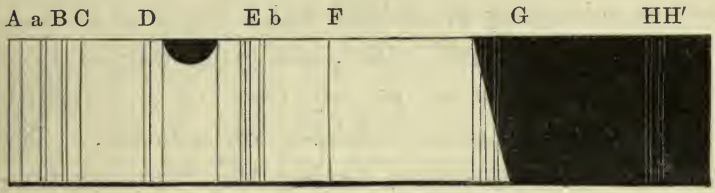
Cerebric acid (Fremy's).—1. Brain is cut into small pieces, treated repeatedly with boiling alcohol, and left for some days in alcohol, to remove water, then pressed, pounded, and extracted with boiling ether, which dissolves cerebric acid and other substances. Distil off the ether, and exhaust the residue with cold ether to remove cholesterine and lecithine (q. v.). Boil with absolute alcohol acidified slightly with sulphuric acid, and filter hot. The deposit formed on cooling must be washed with cold ether to remove oleo-phosphoric acid (q. v.), and the remaining cerebric acid purified by recrystallisation from boiling ether.

2. White crystalline granules, swelling up like starch, but not dissolving, in boiling water, insoluble in cold, soluble in hot alcohol and ether. From the

emulsion in water it is precipitated by neutral salts, such as sodium chloride.

3. Heated on platinum foil it quickly decomposes, burning with a peculiar smell, and leaving a difficultly combustible charcoal, and at last a white residue of phosphoric acid.

4. Treat cerebric acid with concentrated sulphuric acid—a red solution will be formed. Add a little sugar—a deep purple colour will be developed, similar to that obtained with bile acids (q. v.).



Spectrum of cerebric acid reaction.

5. Dissolve in boiling alcohol, and add sufficient alcoholic potash or soda. A voluminous white precipitate (alkaline cerebrate, Fremy) is formed, and on further boiling will partially dissolve. Filter hot—a white body remains on the filter, which after drying dissolves in boiling alcohol, leaving only a scanty yellow resinous residue, and on cooling deposits pure *Cerebrine* (Müller).

6. Fremy's cerebric acid and Liebreich's protagon are probably compounds, or mixtures in atomic proportions, of lecithine and cerebrine, as appears from the above decomposition and the following comparison of their elementary composition.

	Fremy's Cerebric acid.	Liebreich's Protagon.		Müller's Cerebrine.	Diakonow's Lecithine.
C	66·7	66·2	67·4	68·45	64·27
H	10·6	11·1	11·9	11·27	11·40
N	2·3	2·7	2·9	4·61	1·8
P	0·9	1·1	1·5	—	3·8
O	19·5	18·9	16·3	15·67	18·73
	100·0	100·0	100·0	100·00	100·00

Cerebrine (Müller), $C_{17}H_{33}NO_3$.—1. Brain is made into a thin milk with excess of baryta water (or lead acetate solution), heated till it coagulates, the coagulum boiled with alcohol, and the solution filtered hot. The voluminous white flaky precipitate which forms on cooling, is exhausted with cold ether, and recrystallized from boiling spirit as long as any trace of a yellowish resinous body remains insoluble on solution. The product is pure *cerebrine*, having the following properties:

2. White, loose, very light, tasteless and inodorous powder, neutral to vegetal colours, insoluble in water, cold alcohol, and ether. Under the microscope it consists of small, nearly globular particles.

3. Heated on platinum foil, it turns brown, emitting a smell of burnt horn, then melts and burns with a red flame, leaving a black swollen charcoal, which on long heating burns away entirely without residue.

4. It is insoluble in cold or hot baryta, potash or ammonia.

5. Heated with soda lime it evolves ammonia.

6. Unaltered in cold water, it swells up in hot to a thin, light turbid emulsion, permanent on cooling and unchanged by acids, alkalies, or metallic salts. On

evaporation a residue remains, soluble in hot alcohol, and precipitated on cooling as unaltered cerebrine.

7. Hydrochloric, nitric, and phosphoric acids in the cold effect no change. On boiling with hydrochloric the body turns reddish-violet, then brown, decomposing and depositing a brown resin insoluble in acids and alkalies.

8. In cold oil of vitriol it dissolves with a dark purple colour; the solution mixed with water becomes colourless, and deposits a viscid yellowish substance. This reaction resembles that of bile acids.

9. Heated in a retort with nitric acid it evolves red fumes, and gives a clear distillate. From the liquid in the retort drops of yellow oil separate, which solidify on cooling, and, after being freed from nitric acid by washing with water, dissolve in boiling alcohol, and are deposited on standing in white fatty granules. These, after repeated crystallisation, appear as a white waxy mass, tolerably soluble in hot or cold alcohol, or ether, reacting in alcoholic solution as a feeble acid, and consisting, under the microscope, of clear fatty globules without a trace of crystallisation. This compound contains 12.92 % H, and 75.52 % C; hence, probably, all the nitrogen has been removed. Heated on platinum foil it melts readily, and burns with a bright flame and a smell of burning fat.

10. Cerebrine is free from sulphur. When oxydised by fuming nitric acid, or by fusing with potassium nitrate and potash, it gives no phosphoric reactions with any of the tests. When phosphorus is found, it arises probably from contamination with lecithine.

11. It is decomposed at 80° C; hence the analyses must be made on a substance dried at 75°, at which temperature no decomposition occurs.

Cholesterine, $C_{26}H_{44}O$.—1. Powder a human biliary calculus, boil with alcohol and allow to cool; the cholesterine crystallises out in colourless laminæ and rhombic plates.

2. Extract some brain substance with ether, boil the ethereal extract with alcohol, allow to cool; the cholesterine crystallises out, but mixed with potassium cerebrate and phosphate. Treat with ether and evaporate; the cholesterine will crystallise out.

3. Cholesterine is white, tasteless, without smell; it is insoluble in water, it dissolves with difficulty in cold but easily in boiling alcohol.

4. Mix some cholesterine with a little dilute sulphuric acid, warm gently, add gradually some concentrated sulphuric acid: the cholesterine will soften and acquire a deep red colour without evolving any gas.

5. Heat some cholesterine cautiously in an inclined glass tube; it will fuse, boil and distil, and be condensed as a solid crystalline mass in the cold part of the tube.

6. Enclose cholesterine with any free fatty acid in a glass tube, seal by fusion and heat the tube for a long time to 150°. The cholesterine will combine with the fatty acid and form a cholesteride or body similar to the glycerides or ordinary fats.

Cholic acid, $C_{24}H_{40}O_5$,—1. Boil glykocholic or taurocholic acid, or purified bile, with excess of hot con-

centrated baryta water for some time, allow to cool, separate off the crystalline mass and heat it with hydrochloric acid. The cholic acid will then separate as a glutinous resin. Allow to stand, decant the solution of baryum chloride, wash *the resinous mass* well with water, dissolve in boiling alcohol, and leave the solution to crystallise. Separate the *colourless glassy crystals.*

2. Ascertain their slight solubility in water, greater solubility in ether, and great solubility in boiling alcohol.

3. Expose them to dry air under the receiver of the air-pump over sulphuric acid; observe that they *lose water of crystallisation* and disintegrate.

4. Take a small quantity of the acid and treat with a drop of solution of cane-sugar, adding concentrated sulphuric acid until the acid is entirely dissolved. On standing for a short time the mixture will assume a *purple colour.* This test, known as Pettenkofer's, is also produced by the glyko- and tauro-cholic acid, and these yield it probably by virtue of their decomposition liberating cholic acid. See spectrum, p. 74.

5. Boil cholic acid with nitric acid for a long time, and obtain *cholesteric acid* from the mother liquor. As the same acid is obtained from cholesterine by the same process, it is probable that cholic acid and cholesterine have the same radical in common.

Chondrine.—1. Boil cartilage from a young subject in water in an open vessel during twenty-four hours, or in a Papin's digester under pressure at 120° C.

during four hours, and observe that it dissolves, and that a solution of *chondrine* is formed, which on cooling gelatinises.

2. To the hot solution of chondrine add acetic acid, decant the fluid from the *precipitated chondrine*, and mix it with alcohol and ether. The dry product is pure chondrine.

3. Compare a solution of ordinary gelatine with the chondrine solution, and observe that the former is *not precipitated* by acetic acid.

4. To a pure watery solution of chondrine add some chloride or acetate of sodium, then acetic acid, and observe that the precipitation of chondrine *does not take place.*

5. Place a watery or alkaline solution of chondrine in the polaroscope, and observe that it *rotates towards the left;* the alkaline solution the more so, the more alkali it contains.

6. Boil with sulphuric acid and treat the acid liquid as described under *leucine*, and obtain this body. Observe that *no glykokoll* can be obtained.

7. Boil cartilage or chondrine with concentrated hydrochloric acid until a sample after having been made alkaline reduces Trommer's copper solution. Boil the mixture with litharge, filter; to the filtrate add basic lead acetate and ammonia; isolate the precipitate, decompose it with hydrothion, evaporate to a small bulk. The syrup obtained is *fermentescible glucose.*

8. To a solution of chondrine in hot water add alcohol: the chondrine will be precipitated in flakes.

9. Add sulphuric, hydrochloric, nitric, or phosphoric acid, a *precipitate* will at first form, but will *redissolve* in excess of the acid.

10. Add arsenic, oxalic, acetic, tartaric, citric, or lactic acid, or carbonic acid water; a precipitate will fall, insoluble in excess of acid.

11. Add solution of ferrous sulphate, ferric chloride, alum, cupric sulphate, mercurous or mercuric nitrate; a white precipitate is formed in each case.

12. Add solution of potassium ferrocyanide, or mercuric chloride. No precipitate will be produced.

13. Drop a piece of dry chondrine into strong nitric acid and boil. It will turn yellow and finally dissolve.

Chyle.—1. Collect some white chyle from the *chyle vessels of the intestine* of a freshly killed animal by means of finely drawn out glass tubes containing rarefied air. Observe that the chyle coagulates after some minutes or hours, and can be isolated from the tube as a white cylinder of solid matter.

2. Collect some chyle by means of a repetition of this process in a small vessel. Keep this covered to prevent evaporation; observe that the chyle *coagulates* and on standing separates into white fibrine, and into a white turbid serum, of strongly *alkaline reaction.*

3. Collect chyle from the *thoracic duct* by means of a suction syringe, or from living animals by a canula inserted by means of a skilful operation.

4. Observe microscopically that it contains *white* and *red blood-corpuscles* and *fat granules.*

5. Separate the coagulum from chyle and observe that it has all the properties of *fibrine*.

6. Treat the serum with ether; it will not become clear. Add acetic acid or caustic potash to the serum, and the ether will then extract all the fat.

7. The addition of acetic acid in sufficient quantity to the serum of chyle causes a precipitate of *caseine*.

8. Boil the serum acidified with acetic acid and filtered from the caseine, and observe that the *albumen* is precipitated.

9. In the filtrate prove the presence of *peptones* by the reactions indicated under *Chyme*.

10. Evaporate the filtrate to an extract, and precipitate all peptones by absolute alcohol. Evaporate the alcohol from the filtrate. Remove fats by extraction with ether. Then extract by means of ether to which some sulphuric acid has been added. The ether leaves *lactic acid* on evaporation, to be purified and tested as shown under that paragraph.

11. Examine the extract for *glucose* by Trommer's alkaline copper-solution.

12. With the fats extracted under 10 some *urea* is extracted. Separate this by water from the ether residue and test with nitric and oxalic acid.

13. Burn the extract of chyle and analyse the *alkaline ash*. Observe its great similarity to the ash of the serum of blood; it contains little iron and phosphoric acid, with lime and magnesia, but a considerable amount of chlorine and alkalies, in the latter soda prevails.

Chyme.—1. Subject chyme to *dialysis*, and use the dialysed liquid, after evaporation to a small bulk, for the following tests, or

2. Obtain *peptones* from fibrine, albumen, syntonine, or gluten by artificial digestion with pepsine and dilute hydrochloric acid, and use the resulting liquids for these tests.

3. Boil the solution; it is not coagulated.

4. Add pure taurocholic or glykocholic acid; in each case a *precipitate* is immediately produced.

5. Observe that the solutions turn the ray of polarised light *towards the left.*

6. Precipitate the solution with absolute alcohol, the white flakes of *precipitated peptone* are again soluble in very dilute alcohol.

7. Add Trommer's copper solution to chyme or peptone, and observe that it forms a purple fluid. Boil, and if a red precipitate ensues glucose is present in the chyme.

8. Add cupric sulphate, ferric chloride, dilute mineral acids to peptones, and see that *no precipitates* ensue.

9. Add chlorine, iodine, tannin, corrosive sublimate, mercuric or mercurous nitrate, silver nitrate, neutral or basic lead acetate, and observe that the peptones give a precipitate with each of these reagents.

10. Boil peptone with mercurous and mercuric nitrite and nitrate with ultimately a slight excess of nitric acid, and observe that a red precipitate and solution results.

11. Ascertain the composition of any peptone pro-

duced by process 6, to be almost equal to the original substance from which it was produced by artificial digestion.

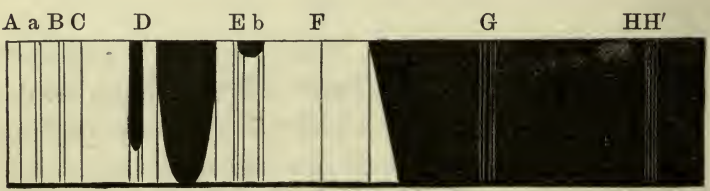
Connective tissue.—1. Boil connective tissue with water. It will swell up and at last dissolve. The filtered solution will have the properties of a solution of *gelatine* (q. v.).

2. Treat with acetic acid; it will swell up and become transparent. Add water and boil. It will dissolve. To the solution add potassium ferrocyanide. No precipitate will ensue.

Corpora lutea. See *Ovario-luteine.*

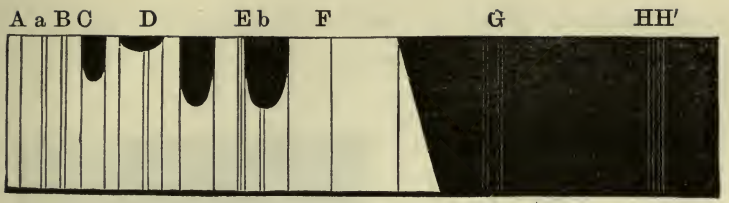
Cruentine.—1. Boil the blood-corpuscles, purified as in the preparation of hæmatine, with sulphuric acid. The brown-red flakes of cruentine which form in the liquid must be washed with water till neutral.

2. Dissolve the flakes in sulphuric acid. The spectrum will show three bands.



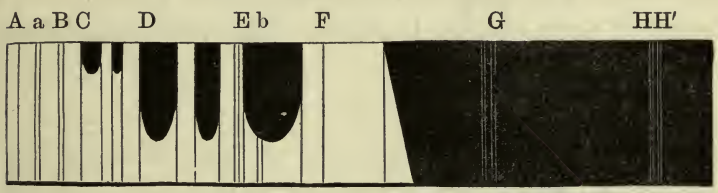
Spectrum of sulphate of cruentine.

3. Treat a portion of the dried flakes with ether or chloroform. The red chloroform solution has a spectrum of four bands and a powerful rose-red fluoresence.



Spectrum of neutral fluorescent cruentine.

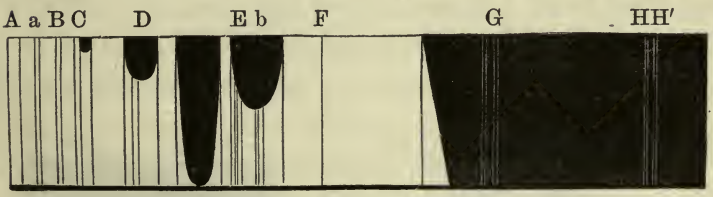
4. Treat a portion of the flakes already extracted with chloroform, with alcohol. It will dissolve almost entirely, and the spectrum of the solution will show five bands.



Neutral five-banded cruentine in alcohol.

Add ammonia till alkaline, one band will disappear and the others slightly change their position and size.

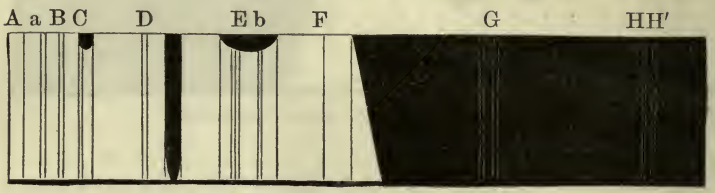
5. Cruentine dissolved in ammonia shows four bands.



Spectrum of alkaline cruentine.

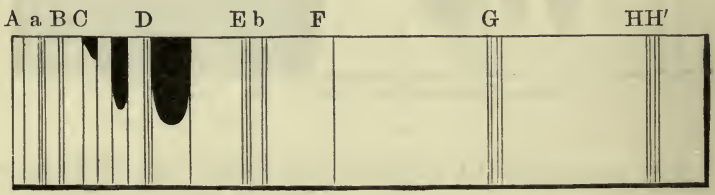
When treated with the ferrous tartrate solution described under hematine, it is reduced and now shows

three bands. Shaking with air re-oxydises it, and restores the former spectrum.



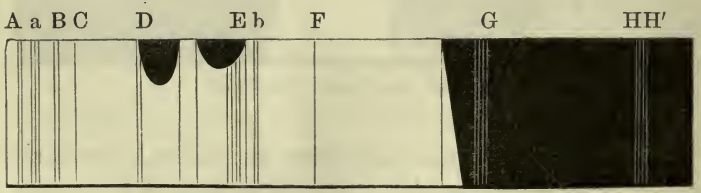
Spectrum of reduced cruentine.

6. Treat the chloroform solution obtained in 2 with hydrochloric acid and water. Warm the solution till clear. The spectrum will show three bands. Add excess of ammonia and warm. The three bands are unaltered.



Hydrochloric product of fluorescent cruentine.

7. Boil the flakes obtained in 1 with much alcohol and filter hot. On cooling red flakes will be deposited. Filter, wash with alcohol, and dissolve them in dilute hydrochloric acid, and observe spectrum of two bands.



Spectrum of cruentine hydrochlorate.

Neutralise with carbonate of sodium, wash precipitate, and dissolve in some carbonate of sodium. Observe that the spectrum has the same two bands as the hydrochloric acid solution.

Cystine, $C_3H_6NSO_2$.—1. A urinary calculus containing cystine (see *Calculi*) is powdered, digested with ammonia, filtered, and the filtrate allowed to evaporate and to crystallise. The crystals must be washed with water. They are insoluble in water and alcohol.

2. Heat a little powdered cystine with potash solution till dissolved, add acetic acid in slight excess. The cystine will be slowly deposited in neutral six-sided laminæ. Examine microscopically.

3. Dissolve a small quantity of cystine in dilute hydrochloric, nitric, or sulphuric acid. Evaporate the solution and set aside to crystallise. Grouped acicular crystals of a cystine salt will gradually be formed.

4. To the solution in acid obtained in the foregoing experiment add ammonium carbonate. The cystine will be precipitated as a white powder.

5. Heat some dry cystine in a tube. It will give off a thick, fetid oil, hydrocyanic acid, and ammonia, and leave a porous charcoal.

6. Fuse with caustic potash in a tube. An inflammable gas will be evolved forming sulphurous anhydride when burned. Dissolve the residue in water and add lead acetate; a black precipitate will appear, proving the presence of sulphur.

8. Heat on platinum foil. A peculiar, disagreeable

odour will be given off, and the cystine will burn off without melting, evolving a smell of hydrocyanic acid.

Dentine.—1. The main portion of the substance of the teeth, covered outside by the enamel. The structure is tubular. It contains animal matter, calcium carbonate and phosphate, and a trace of magnesium phosphate.

2. The *enamel* contains calcium carbonate, phosphate, and fluoride, magnesium phosphate, and a very little organic matter.

Dextrine, $C_{12}H_{10}O_{10}$.—1. Take 20 grammes of starch, mix with 30 c.c. of water. Dilute 5 grammes of concentrated sulphuric acid with 30 c.c. of water and add the dilute acid gradually to the starch paste. Mix well and heat for some time in a water bath at 90° C. When cold add alcohol; the dextrine will be precipitated.

2. Dextrine cannot be crystallised; it is soluble in water, but insoluble in absolute alcohol.

3. Dissolve a portion in water and add a drop of dilute tincture of iodine; the solution remains uncoloured. Starch, under similar circumstances, would be coloured a deep blue.

4. Add to an aqueous solution of dextrine, some solution of caustic potash and a drop of a very dilute sulphate of copper solution; boil; the copper salt will be reduced, the liquid darkens, and finally a bright red precipitate of copper suboxyde is precipitated.

5. It rotates polarised light to the right. Boil dex-

trine with dilute sulphuric acid, it will be converted into *glykose*.

Elastic Tissue.—1. From the neck-band and middle coat of arteries and veins. Remove extraneous matters by boiling with alcohol, ether, water, strong acetic acid, dilute potash, water, dilute hydrochloric acid (10%), and again with water and dry at 100° C. The residue, *elastine*, is a brittle, yellowish, fibrous, mass, swelling up, but insoluble in water, insoluble in alcohol, ether, and acetic acid. Examine as follows:

2. Soak in dilute acetic acid; it will recover its elasticity and fibrous appearance.

3. Boil a long time with water; it will remain insoluble and will not be converted into gelatine. (See *Connective Tissue*.)

4. Digest with strong caustic potash; a brown solution will be formed. Neutralise with sulphuric acid and add tannic acid, a precipitate will ensue. No other acid will produce a precipitate, and the solution will not form a jelly when evaporated.

5. Chemolyse by boiling with dilute sulphuric acid; it will yield nearly the same products as albumen (*q. v.*).

6. Heat on platinum foil; it will burn away entirely without ash.

7. Fuse with caustic potash, dissolve in water, and add potash-acetate of lead. No black precipitate will form, proving absence of sulphur.

Excretine. $C_{78}H_{156}SO_2$ (Marcet).—1. Extract dried human fæces with boiling absolute alcohol, on evapo-

rating the extract and allowing to stand, if possible at 0° C., silky crystals of excretine are deposited.

2. Excretine is very soluble in ether.

Excretolic Acid (Marcet).—1. Obtain, as directed under fæces and purify by dissolving in hot alcohol, and allowing to deposit on cooling.

2. It is an olive-coloured substance with an offensive odour of fæces, very soluble in hot alcohol and in ether, slightly soluble in cold alcohol, insoluble in water. The alcoholic solution has an acid reaction.

3. Heat on platinum foil; it will burn with a luminous flame, evolving a fæcal smell.

Fæces.—1. Dry and ascertain proportion of water (73 to 75%).

2. Exhaust a quantity of dried fæces with boiling alcohol. (The residue will be insoluble in water and ether and will consist mainly of food-remains and insoluble inorganic matters.) Filter, and allow the solution to stand. A deposit containing a peculiar fat called *excretolic acid* (*q. v.*) will gradually form.

3. Filter again, add milk of lime; a brown precipitate will fall. Dry the precipitate and extract it with ether; the ether on evaporation will leave *excretine* (*q.v.*), which may be purified by crystallisation from alcohol.

4. The portion insoluble in ether must be boiled with dilute hydrochloric acid, and the resulting *fatty acid* (generally margaric) washed with water and treated as directed under *fats* (*q.v.*).

5. Another portion of this insoluble matter should be digested with dilute sulphuric acid, and the solution examined spectroscopically for products of decomposition of hematine or other matters.

6. Digest the dried fæces in chloroform: no cholophæine can be extracted, showing that the biliary matters, if present, had been changed in composition in passing through the intestine.

7. Distil the fresh undried fæces with dilute sulphuric acid; a fetid distillate will pass over, containing volatile acids (*q.v.*) and an essential oil.

8. Burn dried fæces and analyse ash. Notice predominance of potash over soda.

Fats. Extraction and separation of animal fats.—1. Exhaust the substance (concentrated to an extract, if liquid) with boiling 90% alcohol containing enough sulphuric acid to decompose any soaps that may be present, evaporate the mixed filtered extracts to near dryness, treat with three or four volumes of ether, allow to stand for some time with frequent agitation, decant or filter.

2. The matter insoluble in cold ether may contain cerebric acid, palmitin, and stearin. Boil with caustic potash solution till saponified, separate out the soaps by adding solid potassium chloride, collect, press, dry at 100° C., reduce to as fine a powder as possible, and digest with cold alcohol. Potassium cerebrate will remain undissolved.

3. Wash the cerebrate with cold alcohol, decompose by boiling alcohol containing sulphuric acid, and

decant or filter. On cooling, the *cerebric acid* will be deposited, and must be washed with cold ether and purified by recrystallising from boiling ether.

4. Warm the alcoholic solution of stearate and palmitate, add cautiously alcohol containing sulphuric acid till all the potassium has been precipitated as sulphate. Filter, evaporate down, and separate the *stearic* and *palmitic acid* by crystallisation from absolute alcohol, in which the former is the less soluble.

5. The ethereal solution must be evaporated to dryness, boiled with caustic potash till saponified, and decanted from undissolved *cholesterine*.

6. The cholesterine is purified by crystallisation from boiling alcohol.

7. The soap, containing *oleic* and *margaric acids*, must be salted out by potassium chloride, again dissolved and salted out, dried, powdered, and digested with cold alcohol, in which oleate is the more soluble. From the salts thus separated the acids are obtained by sulphuric acid as above. The margaric acid may be purified by crystallisation from alcohol. Digest the oleic acid at 100° C. with half its weight of finely-powdered lead oxyde, mix the resulting mass with two volumes of ether, allow to stand; decant from any undissolved matter, shake the solution with enough dilute sulphuric acid to combine with nearly all the lead, separate off the solution of oleic acid in ether, and drive off the ether by distillation.

Fibrine of blood.—1. Beat briskly a quantity of fresh-

drawn blood with a bundle of twigs. Remove the fibrine adhering to the twigs, put it in a bag, tie the mouth of the bag tightly round a tap, and allow water to run through, frequently kneading the mass, until colourless. The substance thus obtained is fibrine mixed with white blood-corpuscles.

2. Keep moist fibrine in a covered beaker in a warm place. It will gradually liquefy and give off an offensive smell, owing mainly to butyrate and valerate of ammonia. The mass mixed with water will coagulate on heating, showing the presence of *albumen*. Ammonium sulphide will also be formed, and will blacken a slip of paper moistened with lead acetate.

3. Extract with boiling water, evaporate to dryness, and digest with strong alcohol. Evaporate the alcohol solution to dryness; the residue will give the reactions of *leucine* (*q.v.*). The portion insoluble in alcohol must be treated with a little concentrated hydrochloric acid, and the acid solution precipitated by excess of strong sodium acetate solution. The precipitate will give the reactions of *tyrosine* (*q.v.*).

4. Dry a portion and heat on platinum foil. It takes fire, giving out a smell of burnt feathers, and leaves a porous charcoal.

5. Dissolve fibrine in dilute caustic potash at 60° C., filter, and add slowly acetic or tribasic phosphoric acid. A precipitate will be formed, soluble in excess of acid.

6. Boil with caustic potash, ammonia will be evolved. Potassium sulphide will be formed in the

liquid and will give a black precipitate with lead acetate.

7. Concentrated hydrochloric acid on warming dissolves it with a violet colour.

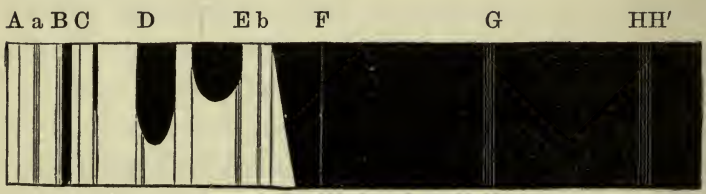
8. Add nitric acid; the fibrine will turn yellow and dissolve.

9. Add concentrated acetic or tribasic phosphoric acid; a gelatinous mass, soluble in water, will be produced.

10. Tannic acid precipitates it from its solutions, and forms with it in the solid state a hard imputrescible mass.

11. Place a portion of fibrine in dilute hydrogen peroxyde; it will become covered with bubbles of disengaged oxygen. Prove that the gas is oxygen.

Fluopittine.—A resinous body obtained from fluorescentine (*q.v.*)., showing the following remarkable spectrum.



Spectrum of fluopittine.

Fluorescentine.—An organic base obtained in decomposing albuminous substances by chemolysis.

Formic acid $CH_2\dot{O}_2$.—1. Obtain from animal substances, as described under *Acetic acid*, 1 and 2. Formic acid boils at 101° C., giving an inflammable vapour.

2. To sodium formiate solution add a little ferric chloride. The same effect will appear as with an acetate.

3. Heat sodium formiate with mercuric chloride solution. A precipitate at first of mercurous chloride and finally of metallic mercury will appear.

4. Add silver nitrate solution and heat. Metallic silver will be deposited as a black powder or a mirror-like coating.

5. Heat a formiate with dilute sulphuric acid. Formic acid, recognized by its odour, will be set free.

6. Heat a formiate with sulphuric acid and alcohol. Formic ether, of a peculiar pleasant odour, will be evolved.

7. Heat a formiate in powder with concentrated sulphuric acid: carbonic oxyde will be given off, and will burn with a blue flame. The mixture will not blacken.

Gastric juice.—1. Gastric juice, obtained through a gastric fistula or by means of the stomach pump or gastric syphon, must be filtered and examined as follows:

2. Boil; no precipitate will be formed. Test the reaction with litmus; the reaction is acid. Notice taste, smell, and appearance.

3. Add alcohol of 90% strength, collect the precipitate of *pepsine* (see pepsine).

4. Distil some gastric juice. The first distillate will be acid, but will give no precipitate with silver nitrate.

The last portions, however, will contain *hydrochloric acid.*

5. Test the juice for *lactic acid* (see Lactic acid).

6. Evaporate a quantity of the juice to dryness, and burn. Examine the ash for phosphates and chlorides, and for potash, soda, lime, magnesia, and iron. More sodium will be found than potassium.

7. Digest pieces of caseine or coagulated albumine in gastric juice; they will dissolve. Boil the solution; it will not coagulate. Add potassium ferrocyanide, lead acetate, dilute alcohol, or a mineral acid; no precipitate will appear. Add solution of tannic acid or mercuric chloride; a precipitate will be formed.

Gelatine, or Osseine.—1. Digest clean bones with hydrochloric acid mixed with nine parts of water. Replace the acid once or twice by fresh more dilute acid, until nothing more is dissolved. Wash the remaining mass repeatedly with water to remove acid, and dry at a steam heat.

2. Treat with cold water; it will swell up but not dissolve. Boil, and a solution will be formed. Use the solution for the following experiments.

3. Add a solution of tannic acid; the gelatine will be precipitated. No other acid will precipitate it.

4. Add solutions of alum, ferric sulphate, potassium ferrocyanide, cupric sulphate, or lead acetate. No precipitate will be produced.

5. Add mercuric chloride solution in excess. A white precipitate will form.

6. Boil one part of dry gelatine with four parts of

sulphuric acid and twelve parts of water for thirty-six hours. Add excess of milk of lime or baryta; boil and strain. Acidify very slightly with sulphuric acid, filter, and evaporate to a low bulk. The granular crystals which form on standing must be removed, boiled with alcohol, filtered, and the solution evaporated to crystallisation. The crystals will consist of glykokoll recognised by the usual tests.

7. Boil gelatine with strong caustic potash till completely decomposed; neutralise with sulphuric acid, and evaporate to a low bulk. From the residue warm alcohol will extract leucine, which may be recognised by the usual tests (see leucine).

Globuline.—1. Pound a number of crystalline lenses with water, filter, evaporate the filtrate in vacuo over sulphuric acid, and wash the product with ether and dilute alcohol. Examine as follows:

2. Treat with water; it will dissolve. Filter and heat the solution. At 73°C it will become opalescent; at 93° it will coagulate.

3. Pass through the aqueous solution a current of carbonic anhydride; a precipitate will fall soluble in pure water.

4. The other reactions are similar to albumine (*q. v.*).

Glycerophosphoric acid, $C_3H_9PO_6$.—1. Obtain *by synthesis* as follows:—Digest glycerine with an excess of glacial phosphoric acid, mix with water, neutralise with baryum carbonate, then with baryta water, filter,

precipitate the baryum with just sufficient sulphuric acid, and evaporate the filtrate in vacuo.

2. *From Brain.* See Brain.

3. *From Lecithine.* See Lecithine.

4. *From Egg-yelk.* Dry in a water bath, extract with boiling alcohol, evaporate to dryness, remove the oil by draining and pressure between paper at a temperature of 80°C, and boil the viscid residue for twenty-four hours with dilute caustic potash. Add to the solution acetic acid in slight excess, and filter; precipitate the filtrate with lead acetate, decompose the washed precipitate with hydrothion, evaporate cautiously, and treat with a little silver oxyde to remove hydrochloric acid, and the filtrate with hydrothion to remove silver. After driving off the hydrothion by evaporation neutralise the liquid with calcium carbonate, filter, and concentrate. Calcium glycerophosphate will crystallise out, and may be purified by recrystallisation. The acid may be isolated by precipitation with oxalic acid and evaporation in vacuo.

5. It is a very acid, gummy, *uncrystallisable* liquid, very soluble in water and alcohol. The somewhat concentrated aqueous solution decomposes by heating into phosphoric acid and glycerine.

6. Heated on platinum foil it burns with difficulty, leaving a charcoal containing *phosphoric acid.*

7. The baryum salt is very soluble in water, and is precipitated by alcohol.

8. The calcium salt crystallises in white pearly scales, of the formula $C_3H_7CaPO_6$, decomposes above 170°C. Boiling with lime water resolves it into

calcium phosphate, and glycerine. It is more soluble in cold than in hot water, and is precipitated by alcohol.

9. The lead salt is insoluble in water and alcohol.

Glykocholic acid, $C_{26}H_{45}NO_6$.—1. Add to some fresh ox-bile a solution of neutral lead acetate, filter off the precipitate, heat it with boiling alcohol, filter hot, pass hydrothion into the filtrate while hot, and filter off the sulphide of lead. Allow the filtrate to cool; it will solidify into a white crystalline mass of *glykocholic acid*, which, after washing with water, is perfectly pure.

2. Add to a small granule of glykocholic acid or of its salts a drop of cane sugar solution, and then concentrated sulphuric acid in drops, agitating the solution, and preventing overheating; a violet or purple liquid will be formed.

3. To glykocholate of sodium dissolved in absolute alcohol add ether; a glutinous precipitate will ensue, which on standing under the ether alcohol will transform into crystals.

4. To a glykocholate dissolved in water add hydrochloric acid; a glutinous deposit of amorphous glykocholic acid will ensue.

5. Boil some glykocholic acid with excess of baryta water; *cholate* of baryum will crystallise after cooling, and *glykokoll* will remain in solution to be extracted as stated under that substance.

Glykogen, $C_6H_{10}O_5$ (*Hepatine*).—1. Mince a fresh liver and boil with a small quantity of water. Strain

and press. Precipitate the filtrate with four or five times its volume of 90% alcohol. Yellowish white flocks will be precipitated. These are well washed with alcohol and then boiled for half an hour or an hour with solution of caustic potash; dilute the solution with a small quantity of water and add four or five times its volume of 90% alcohol. The glykogen is precipitated and, by washing with alcohol is obtained tolerably pure.

2. Mince a liver and boil, &c., as before, allow the extract to cool, and add glacial acetic acid, when the glykogen will be precipitated; filter off and dry. Glykogen is a white mealy powder; it exhibits no definite structure under the microscope.

3. It is soluble in water, insoluble in alcohol.

4. Boil glykogen with some dilute hydrochloric acid, it will be transformed first into dextrine and finally into grape sugar. This transformation can be detected by the reducing action of sugar on alkaline copper solution. (See Dextrine, &c.)

5. Boil an aqueous solution with a solution of caustic potash to which a drop of dilute solution of copper sulphate has been added. The copper salt will not be reduced.

6. Add to an aqueous solution a drop of dilute tincture of iodine; a violet-red colour is produced.

Glykokoll, $C_2H_5NO_2$. — 1. Boil glue with caustic potash. Ammonia is evolved in large quantity. Add sulphuric acid till the liquid is neutral; evaporate,

separate the potassium sulphate, which will crystallise out; evaporate the clear liquid to dryness. Exhaust the residue with hot alcohol; allow to cool; the glykokoll will crystallise out.

2. Boil hippuric acid for half an hour with strong hydrochloric acid, dilute the liquid with water, and allow to cool. The benzoic acid will separate in crystals; decant the clear liquid, treat with ammonia, and evaporate on a water bath; wash the dried residue with alcohol; the glykokoll is left in a crystalline powder.

3. Glykokoll crystallises in hard granular crystals; it has a sweet taste.

4. It dissolves with difficulty in water; it is insoluble in absolute alcohol.

5. Pass nitrous acid into a solution of glykokoll. The whole of the nitrogen is evolved, and glykollic acid ($C_2H_4O_3$) is formed soluble in ether. Shake the liquid with some ether; allow to stand; draw off the ethereal layer with a syphon; evaporate, when the crystallised glykollic acid is left.

6. Boil an aqueous solution of glykokoll with copper oxyde; a copper salt is obtained, which crystallises out in blue crystals, turning green when dried at 100°C.

Glykose or Glucose, $C_{12}H_{24}O_{12}$.—1. Boil twenty grammes of starch with eighty c.c. of water, add one to two grammes of concentrated sulphuric acid, and boil for twenty-four hours, adding water as the solution evaporates. Add calcium carbonate to neutralise the free acid, allow to stand, decant, and evaporate the

decanted liquor to a thin syrup, from which in a few weeks the glucose will crystallise out.

2. Heat some glucose with a solution of bichromate of potash and sulphuric acid in a test tube furnished with a cork and delivery tube; pass the evolved vapours into a solution of nitrate of silver; formic acid will be evolved, which will reduce and blacken the silver solution.

3. Add some cold concentrated sulphuric acid to glucose; the latter will dissolve without blackening; cane sugar blackens.

4. Dissolve some glucose in water, add caustic potash solution, and a drop or two of very dilute copper sulphate solution; after long standing in the cold, or immediately on boiling, the copper salt will be reduced, and a bright red precipitate of copper suboxyde falls.

5. Add a solution of glucose to a solution of silver nitrate and heat gently; the silver salt is reduced, and metallic silver is deposited either as a black powder or as a mirror-like coating.

6. Dissolve two parts of ferricyanide of potassium and one part of hydrate of potassium in water, warm, and add aqueous solution of glucose. The solution will be decolorised. Neither cane sugar nor dextrine give this reaction.

7. To an aqueous solution of glucose add a concentrated solution of common salt, and allow to stand; crystals of chloride of sodium and glucose separate. These crystals will be formed if diabetic urine be evaporated and allowed to stand.

8. Diabetic sugar is identical with dextro-glucose above described.

Guanine, $C_5H_5N_5O$.—1. Boil 500 grm. of Peruvian guano with milk of lime till the liquid has turned from brown to greenish yellow, filter, neutralise with hydrochloric acid, and allow to stand some hours. Wash the deposit, treat with boiling hydrochloric acid, decant the clear liquid from the undissolved uric acid, set aside to crystallise, and purify the crystals by recrystallising from HCl. Finally precipitate by ammonia, wash with water and dry.

2. Pure guanine is a white, neutral amorphous powder, insoluble in water, soluble in strong acids and alkalies. The hydrochlorate crystallises in thin, light yellow needles, which lose water at 100° C., and acid at 200° C.

3. Dissolve a little guanine in hot nitric acid. On cooling, long, very fine, interlaced crystals of nitrate will form.

4. Treat solution of guanine hydrochlorate with ammonia oxalate. Crystals of guanine oxalate will be produced.

5. Mix hot saturated solution of guanine in HCl with excess of hot concentrated $PtCl_4$, evaporate to one half and allow to cool. Wash with alcohol and ether the yellow needles formed, and dry in vacuo over sulphuric acid. Weigh a small portion, ignite in a weighed crucible, and weigh the residue of spongy platinum. The salt should contain 35·17 of platinum. Formula $C_5H_5N_5O$, $HCl+PtCl_4+2H_2O$.

Hair.—1. Notice structure under microscope.

2. Digest with concentrated warm hydrochloric acid; it will very slowly dissolve to a violet solution.

3. Heat with nitric acid, it will turn yellow and in great part dissolve.

4. Boil with strong caustic potash, it will dissolve. Add to the solution potash-lead acetate, a black precipitate of sulphide will ensue, proving the presence of sulphur.

5. Heat on platinum foil; it will burn with an odour of horn and leave a swollen charcoal, and finally about 1% of ash containing iron.

6. Chemolyse by means of sulphuric acid. Leucine, tyrosine, &c., will be obtained.

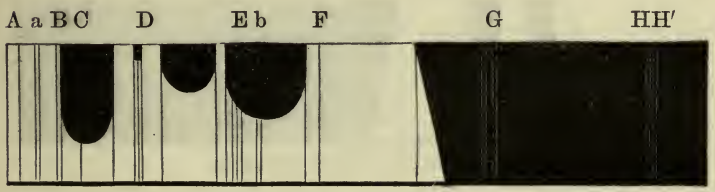
Hematine.—1. Dilute one volume of saturated sodium chloride solution with fifteen volumes of water. Add one volume of blood, freed from fibrine by beating and filtered through a cloth. Mix well and allow to stand in ice and water till the corpuscles have settled. Decant the liquid and wash them with the same quantity of sodium chloride as before. Repeat this operation a third and fourth time. Shake the corpuscles with water and ether, separate off the ether, and to the red watery solution add basic lead acetate solution in slight excess, filter off the precipitate, and remove excess of lead by a little potassium carbonate. To the filtrate add potassium carbonate in powder till the colouring matter separates, filter off the flakes, press them strongly, break the mass, and exhaust with strong alcohol. To the extract add alcoholic solution of tar-

taric acid to a slightly acid reaction, filter, and evaporate at 60° C. to one tenth of its bulk. On standing minute crystals of hematine are deposited, and may be purified by washing successively with ether and cold water.

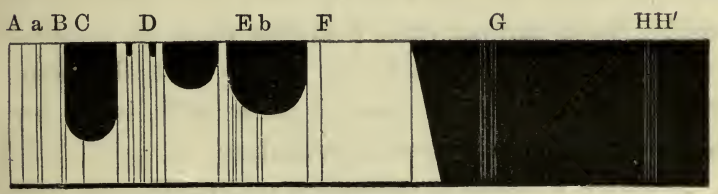
2. Examine the hematine under the microscope, it will be found to consist of rhombic crystals.

3. Hematine is insoluble in water, alcohol and ether when neutral, but soluble in water containing caustic alkali, or in acid or alkaline alcohol.

4. Dissolve some hematine in alcohol and a little sulphuric acid. The spectrum will show four, under

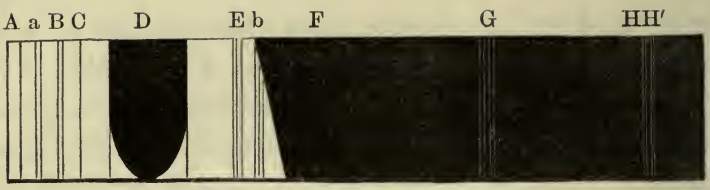


Spectrum of four-banded hematine.



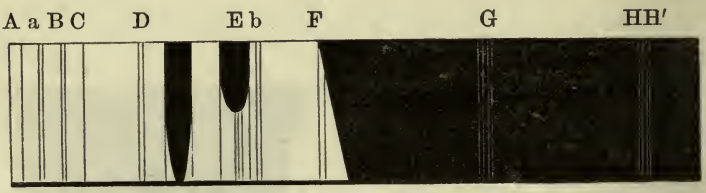
Spectrum of five-banded hematine.

certain circumstances five, bands. Render the solution alkaline by caustic potash. The spectrum will show only one broad band. Acid will restore the spectrum.



Spectrum of pure alkaline hematine.

5. Dissolve hematine in water with a little caustic potash. To a solution of ferrous sulphate add tartaric acid and then ammonia till alkaline. Pour a little of the clear mixture into the hematine solution, and examine the spectrum. It will show two bands. Shaking with air will restore the former spectrum.



Spectrum of reduced hematine.

Hippuric Acid, $C_9H_9NO_3$.—1. Evaporate the fresh urine of horses or cows which have been kept in the stable for a day or two previous to $\frac{1}{6}$ or $\frac{1}{8}$ of its original volume, and add an excess of hydrochloric acid. After standing the hippuric acid is thrown down as a yellowish-brown precipitate.

2. Filter or decant the liquid and purify the crude hippuric acid as follows:—Boil with milk of lime and filter; precipitate the filtrate with sodium carbonate, boil, filter, add solution of chloride of lime, filter, add an excess of hydrochloric acid. The hippuric acid will finally be precipitated in a colourless condition.

3. Prepare from urine thus :—Evaporate it to a syrup, add some hydrochloric acid, and shake in a stoppered bottle with its own volume of ether; allow to stand one hour, then add $\frac{1}{20}$ of its volume of alcohol; allow to stand a short time; remove the upper ethereal layer with a syphon. Shake this ethereal extract with a small quantity of water; allow to stand; remove the ethereal layer as before and evaporate. The hippuric acid will be obtained in crystals usually of a yellowish-brown colour. This colour can be removed by treatment with animal charcoal.

4. Recrystallise the acid from boiling water, in which it is easily soluble. Observe its slight solubility in cold water, and easy solubility in alcohol.

5. Melt it in a glass tube and let it consolidate by cooling. Then heat again to fusion and dry distillation. Observe the irritating vapours of benzoic acid, and a sublimate of the same, and a charcoal left in the bottom of the tube.

6. Boil hippuric acid for half an hour with hydrochloric acid. It will be split up into glykokoll and benzoic acid. Separate both as described under glykokoll.

7. To a solution of neutral hippurate add solution of ferric chloride. A cinnamon-coloured precipitate of ferric hippurate will be formed.

Hypoxanthine or *Sarkine*, $C_5H_4N_4O$.—1. The mother liquor of xanthine hydrochlorate, obtained as under Xanthine, is evaporated, and another crop of crystals removed (sarkine salt containing xanthine). The

mother liquor of this will, on further evaporation, yield nearly pure sarkine salt. Purify by recrystallisation, dissolve in hot caustic potash, add ammonium chloride, filter if necessary, pass carbonic acid in excess through the solution, collect, and wash the precipitate of *sarkine*.

2. Its reactions are those of xanthine, except its greater solubility in hydrochloric acid, and the following:

3. To a *dilute* solution of hypoxanthine in nitric acid add silver nitrate; a copious white precipitate will be produced (see Xanthine 5).

Inosic acid, $C_5H_8N_2O_6$.—1. Take the mother liquor obtained in the preparation of kreatine (see Kreatine) from flesh. Concentrate and mix with alcohol till it has a milky consistence, allow to stand and crystallise. Pour off the liquid, dissolve the crystals in hot water, add a solution of baryum chloride; crystals of baryum inosate are deposited on cooling. Separate these crystals, dissolve in a little boiling water, and add sulphuric acid as long as a precipitate falls; allow to stand, decant, and evaporate the clear liquid, when inosic acid will be left as an uncrystallisable, syrupy mass.

2. Inosic acid is easily soluble in water; alcohol precipitates it from aqueous solution; it is insoluble in ether.

3. Add to an aqueous solution of inosic acid a solution of copper sulphate; a bluish-green precipitate falls soluble in ammonia.

4. Heat a portion of inosic acid, or an inosate on platinum foil; it will decompose, giving off the odour of roast meat.

Inosite, $C_6H_{12}O_6$.—1. Mince the muscular portion of the heart; add water, allow to stand for some time, frequently stirring the mass. Separate the residue and press. Boil the filtrate with a little acetic acid, evaporate to one tenth of its bulk, precipitate with a solution of neutral lead acetate, and filter. Mix the filtrate with basic lead acetate: a precipitate falls of impure inosite. Wash the precipitate, and decompose by passing sulphuretted hydrogen; filter off the precipitated lead sulphide. Evaporate the filtrate to a low bulk; separate any crystals which may be formed, and mix the clear liquid with alcohol until a turbidity is produced: *crystals of inosite* will be deposited.

2. Inosite dissolves in water; it is insoluble in absolute alcohol and ether; it has a sweet taste.

3. Heat inosite on some platinum foil; it melts, swells up, evolving gas which burns with a pale blue flame; it then chars and burns with a luminous flame.

4. Evaporate a solution of inosite in dilute nitric acid nearly to dryness. Moisten the residue with ammonia and a little calcium chloride solution; evaporate, when a rosy-red substance will be left.

5. Boil an aqueous solution of inosite with potash containing a drop of dilute copper sulphate; the copper salt is not reduced.

6. Inosite is not altered by boiling with dilute acids or alkalies.

Kreatine, $C_4H_9N_3O_2$.—1. Take finely-chopped meat free from fat, or chopped cod-flesh; mix well with an equal weight of water, squeeze in a bag of strong linen. Boil the extract, filter off the coagulated albumen and myochrome; treat the filtrate with baryta water until it has a strongly alkaline reaction; filter, evaporate on the water-bath to a syrup, allow to stand in a warm place. The liquid will spontaneously evaporate and deposit crystals of kreatine, which can be purified by boiling with animal charcoal and by recrystallisation.

2. Observe the insolubility of kreatine in cold alcohol, and its slight solubility in cold water.

3. Boil some kreatine with a large excess of baryum hydrate; it is decomposed, and ammonia is evolved, the vapour turning red litmus paper blue. This is due to the formation and decomposition of *urea* on the one, and the formation of *alanine* on the other hand; both substances can be obtained from the fluid.

4. Dissolve some kreatine in some hydrochloric, sulphuric, or nitric acid; evaporate at a gentle heat; crystals are deposited which are soluble in alcohol, and consist of a compound of the acid employed with *kreatinine*.

5. Dry crystallised kreatine in a current of hot air, and observe that it loses water of crystallisation.

Kreatinine, $C_4H_7N_3O$.—1. Take fresh human urine, not less than four litres, neutralise it with milk of lime or hot saturated baryta water; add a solution of calcium chloride as long as a precipitate falls. Filter

and evaporate to a syrup, allow to stand, separate the deposited crystals, and add to the syrup one twenty-fourth of its weight of a neutral syrupy solution of zinc chloride. Allow to stand three or four days; the double chloride of zinc and kreatinine is deposited in crystalline, warty masses. Wash this deposit with water.

2. Dissolve it in boiling water; add hydrated oxyde of lead until the fluid has an alkaline reaction. Now add three times as much oxyde of lead as has been already used, and boil the whole for some time; filter. Boil the filtrate with some good animal charcoal, filter, evaporate the filtrate to dryness; a crystalline residue will be left. Boil the crystals with eight to ten times their weight of alcohol, allow to cool, decant the clear liquid from any crystals of *kreatine* which may be deposited, and evaporate it, when crystals of *kreatinine* will be deposited.

3. Treat fresh urine with neutral lead acetate as long as a precipitate is produced; evaporate the filtrate to one quarter of its bulk, and treat with hydrothion to remove excess of lead. Expel hydrothion by boiling, and add corrosive sublimate to the liquid. A white precipitate of kreatinine mercuric chloride will fall. Filter, wash, and decompose with hydrothion; evaporate filtrate to crystallisation, when kreatinine hydrochlorate will be obtained.

4. Place a crystal of kreatinine on a slip of moist turmeric paper; it will produce a brown spot; it has, therefore, an alkaline reaction.

5. Add to a solution of corrosive sublimate a solution

of kreatinine; it will produce a white curdy precipitate, which is soon transformed into needles.

6. Add to an aqueous neutral solution of zinc chloride some kreatinine solution; a crystalline precipitate is immediately produced in the form of warty grains. $2(C_4H_7N_3O)ZnCl_2$.

Kryptophanic acid, $C_5H_9NO_5$, or $C_{10}H_{18}N_2O_{10}$.—1. Evaporate fresh urine to one third; mix with excess of milk of lime or baryta, allow to stand a few hours and filter: acidify the filtrate with acetic acid, evaporate to a syrup, and set aside to crystallise. Separate the crystals by draining and pressure from the mother liquor, add to the latter five times its volume of alcohol of 90%, shake well, allow to stand for five minutes, pour off the liquid and wash the precipitate with a little more alcohol. Warm the sticky deposit to drive off the adhering alcohol, dissolve in a small quantity of water and filter. To the filtrate add twice its bulk of saturated solution of lead acetate, filter from the dark brown precipitate, mix the filtrate with three volumes of 90 % alcohol, wash with alcohol the nearly white precipitate of *lead kryptophanate*, lastly wash with a little absolute alcohol, dry at 100° C., and weigh.

2. Mix with water to a thin cream, and for every 100 parts of the dry salt add an equal weight of sulphuric acid containing 25% of H_2SO_4. After standing for some time, with frequent agitation, filter and test a portion of the filtrate for sulphuric acid; if any be present precipitate it out exactly with baryta water,

adding no excess, and filter. Evaporate the filtrate to a thick syrup, precipitate with 90% alcohol, wash the precipitate with a little more alcohol, and dry the pure kryptophanic acid at a very gentle heat, or in vacuo.

3. The acid is very soluble in water, nearly insoluble in alcohol, insoluble in ether.

4. Dissolve a portion in water : the solution has a pleasant acid taste. Use it for the following experiments.

5. Add a little saturated lead acetate solution: a white precipitate will fall, redissolved by excess, and again precipitate by alcohol.

6. Add copper acetate: a green precipitate will form, behaving like the lead compound.

7. Dry a portion of the green precipitate at 100° C., and distil with a small quantity of water. The substance will turn dark green, and alcohol will be found in the distillate, proved by a diminished sp. gr. and by its reducing a drop of potassium bichromate and sulphuric acid.

8. Add copper sulphate in excess, then excess of caustic potash : if *on long boiling* the copper is reduced, the acid is yet impure; the pure acid does not reduce copper solution.

9. Add mercuric nitrate, or acetate, or silver nitrate. A white precipitate will fall, soluble in nitric acid.

10. Add ammonio-nitrate of silver : the solution will become dark and will gradually deposit black metallic silver.

11. Heat a kryptophanate in the solid state on platinum foil: acid vapours are perceived but no urinary smell, and a residue of difficultly combustible charcoal remains.

12. Add baryum or calcium chloride: no precipitate. Add ferric chloride: a brown precipitate will fall, soluble in excess or in ammonia, deposited again on boiling.

Lactic acid, $C_3H_6O_3$ (from milk).—1. Take 300 grammes of sugar, 3 grammes of tartaric acid, 400 grammes of sour milk, 25 grammes of old cheese, and 150 grammes of precipitated chalk, and 1250 cc. of water; allow to stand in a moderately warm place, about 36° C., from ten to twelve days. Boil the semisolid mass of calcium lactate thus produced with a litre of water and 3 grammes of unslaked lime; filter while hot and gently evaporate the filtrate. Calcium tartrate is gradually deposited in granules. Collect the crystals and press between filter paper. Dissolve the crystals in twice their weight of water, add $\frac{7}{32}$ part of sulphuric acid and filter while hot; add to the filtrate $\frac{3}{10}$ of zinc carbonate, boil for a quarter of an hour, separate the crystalline zinc salt, dissolve in seven to eight times their weight of boiling water, pass in sulphuretted hydrogen, filter and evaporate to a syrupy condition.

2. Boil some lactic acid with nitric acid; the lactic will be converted into oxalic acid: neutralise the liquid with ammonia, add acetic acid and a little calcium chloride solution; calcium oxalate will be precipitated.

3. Lactic acid forms numerous crystalline salts which are mostly but sparingly soluble in water; they are prepared by boiling the acid with a carbonate.

4. *E.g.* Boil lactic acid with zinc carbonate, filter hot; the solution will on cooling deposit crystals of zinc lactate.

5. Similarly boil lactic acid with calcium carbonate, when calcium lactate will be formed.

6. Heat the syrupy acid: it will give off water, and be converted into the anhydride, which fuses below 100° C.; when further heated to 260° C. it is converted into *lactide*, which sublimates in white crystalline plates.

Lecithine, $C_{42}H_{84}NPO_9$.—1. *Preparation of Lecithine (from egg-yelk)*. Extract the yelk with a mixture of ether and alcohol, distil off part of the ether from the extract, add alcohol as long as turbidity ensues from the separation of a fatty oil, and mix the clear yellow solution with platinic chloride acidified with hydrochloric acid. A copious yellow flocculent precipitate of a double salt will be formed, differing greatly from choline-platinic chloride, insoluble in water, easily soluble in ether, carbon disulphide, chloroform, and benzol, precipitated from these solutions by alcohol in yellow flakes agglutinating on agitation. To free it from fat, it must be dissolved five or six times in ether and precipitated each time by alcohol. In vacuo over sulphuric acid it dries without losing its solubility in ether, but at 100° C. it blackens, melts, and loses

weight, as much as 5% in two hours. Its formula is $(C_{42}H_{83}NPO_8Cl)\ PtCl_4$.

2. Cadmium chloride may be used instead of platinic chloride. The yellow flaky precipitate may then be washed with alcohol and ether (in which it is little soluble), and can be more easily freed from fat than the platinic chloride compound. It is soluble in alcohol, containing hydrochloric acid.

3. From these compounds, after removing the metals by hydrothion, lecithine hydrochlorate is obtained on evaporation as a waxy mass. The chlorine having been removed by silver oxyde, and the silver by hydrothion, a homogeneous translucent residue of *free lecithine* remains.

4. *From brain, &c.*, it may be extracted by the process given under *Cerebric acid* and *Brain* (*q.v.*), and purified by the methods described above.

5. Lecithine and its compounds are very easily decomposed. The ethereal solution of the double platinum salt forms on standing a light yellow deposit of *choline-platinic chloride*. The alcoholic solution of lecithine hydrochlorate gives, on long standing, *oily drops*, free from nitrogen and phosphorus, and forming a soap with alkalies. Free lecithine also decomposes, slowly in the cold, rapidly on warming. Boiling with water does not decompose it (Gobley), but addition of dilute mineral acids or alkalies causes decomposition, with separation of *oleic*, *margaric*, and *glycerophosphoric acid*.

6. When lecithine hydrochlorate is poured into boiling baryta water, a smeary baryum salt separates,

while the filtrate contains *choline* and a part of the glycerophosphoric acid. Remove free baryta by carbonic anhydride, and extract the residue with alcohol: *choline hydrochlorate* dissolves, and may be precipitated by platinic chloride, and the yellow flaky precipitate washed with alcohol. By crystallisation from water it is obtained in yellow prisms or tables, closely resembling the choline-platinic chloride prepared from bile. On evaporation the watery solution gives the same crystals down to the last drop. It contains 31·68% of platinum, pointing to the formula 2 ($C_5H_{14}NOCl$) $PtCl_4$, which is the same as that of the compound from bile.

7. To establish the presence of glycerophosphoric acid, the residue insoluble in alcohol is redissolved in water, freed from baryum by sulphuric acid, saturated with calcium carbonate, the sulphuric acid exactly precipitated by baryum chloride, and the filtrate evaporated down; the crystalline calcium glycerophosphate which separates, after washing with alcohol and drying at 100°, gives results corresponding with the formula $C_3H_7CaPO_6$.

8. The baryum salt, insoluble in water, is decomposed by boiling with hydrochloric acid, when a fluid stratum, nearly solid on cooling, separates, and may be washed with water. The liquid contains much baryum chloride, also glycerophosphoric acid, partly decomposed into phosphoric acid and glycerine during the boiling.

9. The *fatty acids* dissolve in ammonia and are precipitated as lead salts by lead acetate. From the

precipitate ether extracts a considerable quantity of *lead oleate*. The oleic acid is purified by crystallising the baryum salt from boiling alcohol; and decomposing the pure salt by hydrochloric acid.

10. The lead salts, insoluble in ether, are decomposed with hydrochloric acid and the free acids crystallised from alcohol. They are finally obtained in white glistening plates, melting at 56·7° C., corresponding in composition with *margaric acid*, but containing probably a little *stearic*, though Heintz's process of fractional precipitation with magnesium acetate fails to effect a separation.

11. Add alcoholic potash to an ether-alcohol solution of lecithine, and observe the precipitation of a crystalline *potassium salt*. Hence lecithine, like glykokoll, is at once *acid* and *base;* it is also, moreover, *a fat*.

Leucic acid, $C_6H_{12}O_3$. — 1. Pass nitrous acid gas through a warm aqueous solution of leucine, add a small quantity of leucine, evaporate to a syrup. Extract the syrupy residue with ether. Evaporate the ethereal solution, when the *leucic acid* will crystallise out. Press the crystals between blotting paper, when nearly pure leucic acid is obtained.

2. Leucic acid is soluble in water, alcohol, and ether. It crystallises in colourless needles. Heat a portion of leucic acid on a watch glass placed on a water bath; the acid will melt and volatilise, the sides of the glass becoming fringed with crystals of the *sublimed acid*.

3. Add to a solution of leucic acid a solution of copper acetate; green flocks of *copper leucate* will be precipitated, sparingly soluble in water, easily soluble in boiling alcohol.

4. Add to a concentrated solution of leucic acid a solution of lead acetate; a white flaky precipitate of lead leucate falls. Boil: part of the precipitate dissolves, the rest melts into a white mass, which becomes hard and brittle on cooling.

Leucine, $C_6H_{13}NO_2$.—1. Boil one part of cowhorn shavings with four parts of concentrated sulphuric acid and twelve parts of water for six hours, renewing the water as it evaporates. Add an excess of milk of lime, boil for some hours, strain, and press. Mix the filtrate with a very slight excess of sulphuric acid, and evaporate the filtrate. Spherical crystalline tufts of *tyrosine* will be first deposited. Separate these, and continue the evaporation, when *leucine* will be deposited in scales.

2. Collect these scaly masses of impure leucine, press them between filter paper. Triturate them in a mortar with slightly warm concentrated nitric acid until dissolved; dilute the solution with water, and add to it solution of mercuric nitrate as long as a precipitate falls; filter, treat filtrate with sulphuretted hydrogen; filter, neutralise the filtrate with ammonia, and evaporate till the leucine begins to crystallise out; allow to cool, when nearly pure leucine will be deposited.

3. To obtain it perfectly pure dissolve in boiling

water, treat with pure and good animal charcoal, filter, and evaporate filtrate till a pellicle begins to form, then pour the boiling hot solution into three or four times its volume of absolute alcohol. Allow to stand, collect on a filter, and press between filter paper; perfectly *white leucine* is thus obtained.

4. Leucine is sparingly soluble in cold, but moderately soluble in hot water, almost insoluble in alcohol; it dissolves in acids and alkalies.

5. Heat gently a portion of leucine in a wide test tube, it will sublime in snow-white flocks. Heat some leucine on a piece of platinum, it will take fire and burn with a white flame.

6. Heat some leucine mixed with soda lime; a strong smell of ammonia is evolved, proving the presence of *nitrogen*.

7. Dissolve leucine in water, pass nitrous acid gas; *leucic acid* is formed.

8. Add to a solution of leucine a concentrated solution of sulphate of copper gradually until an excess of copper is present; the fluid takes a deep blue colour. Treat the deep blue solution with an excess of baryum carbonate, and filter; on evaporation the compound of *copper and leucine* is deposited in blue crystalline granules.

Luteine.—I. *From corpora lutea of Mammals.*

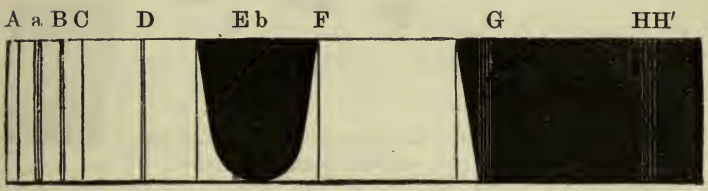
1. Dissect out the corpora lutea from the ovaries (of cows), pound the corpora, warm, and press out the juice.

2. *Reactions of the juice.*

a. Add nitric acid; a precipitate, pink at first, afterwards greenish-yellow, appears.

b. Add sulphuric acid; a precipitate will be formed, which will redissolve and turn reddish-brown.

c. Add sulphuric acid and a little sugar; a fine purple colour will be produced, showing in the spectroscope one band in green.



Spectrum of juice of corpora lutea treated with sulphuric acid.

d Add hydrochloric acid; no reaction.

3. *Reactions* of Alcoholic Extract.

a. Dry the solid residue of the corpora, extract two or three times with boiling alcohol of 85%, filter hot, allow the mixed extracts to stand in the dark till clear, decant the liquid, and examine as follows:

b. Add water; a turbidity will be produced, not removable by heating.

c. Add potash solution; a yellow precipitate will gradually deposit.

d. Add mercuric chloride, auric chloride, or platinic chloride; no reduction will take place.

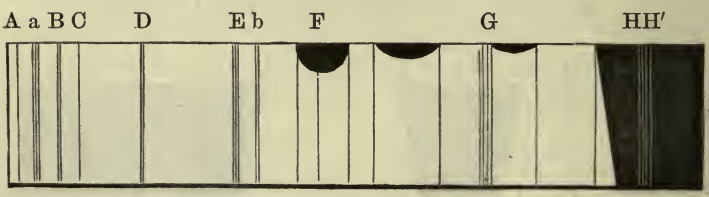
e. Add mercuric nitrate; a copious yellow precipitate will fall, becoming white on heating.

f. Add mercuric acetate; a yellow precipitate will fall, leaving the liquid colourless.

g. Add copper acetate; green precipitate.

h. Add lead or zinc acetate; on standing all the yellow matter will go down as an oily layer.

i. Examine with spectroscope with lime light. Two bands in blue and a third feeble one in violet will appear.

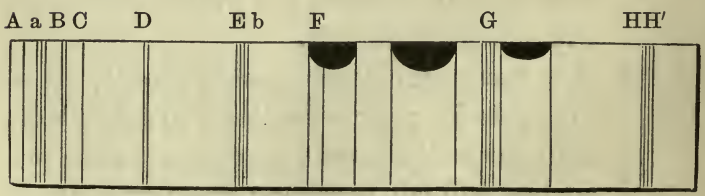


Spectrum of ovario-luteine in alcohol.

4. Set aside the alcohol solution to evaporate spontaneously. The fatty residue will gradually deposit orange-red crystals of *ovario-luteine.* Shake the mixture with absolute alcohol till the fat is removed, then with repeated small quantities of ether. Lastly, wash with ether, press between paper, and dry.

5. *Reactions of ovario-luteine crystals.*

a. The crystals are soluble with a yellow or orange colour in hot alcohol, ether chloro-



Spectrum of ether solution of corpus luteum.

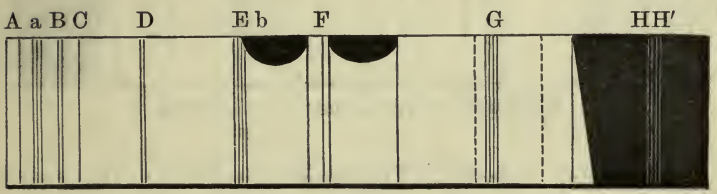
form, carbon disulphide, and hot glacial acetic acid.

b. To a small quantity of the crystals add nitric acid; a blue colour, turning rapidly yellow, is produced. The acetic acid solution gives the same reaction.

6. *Reactions of Chloroform Solution.*

a. Dry the solid residue of the corpora lutea, and extract with chloroform. Test the solution as follows:

b. Examine with spectroscope with lime light. Two bands in blue will be shown.



Spectrum of chloroform solution of corpora lutea.

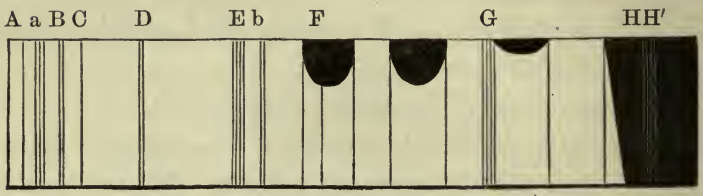
c. Add nitric acid; the solution will become colourless.

d. Evaporate the solution in a current of air. A peculiar odour will be noticed, and a yellow residue left. To the residue add sulphuric acid; a dirty green colour will appear. Add a little sugar; no purple colour will ensue.

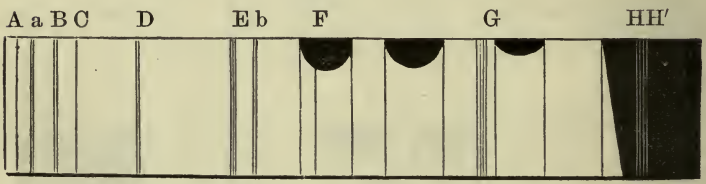
II. *Ovo-luteine, or luteine from egg-yelks.*

1. *Alcohol extract.*—Boil the yelk with 85% alcohol, filter, and allow to stand till clear. It

will show the same three bands as the ether solution, but diminished in intensity by heat, and recovering the same appearance when cold.

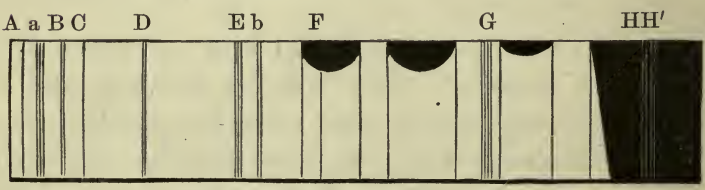


Spectrum of egg luteine in alcohol, cold.



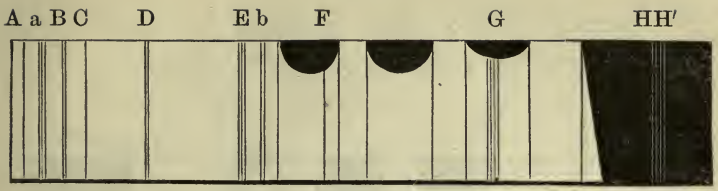
Spectrum of egg luteine in alcohol, hot.

2. *Ether extract.*—Prepared similarly; the ether extract shows three bands in a slightly different position.



Spectrum of egg luteine in ether.

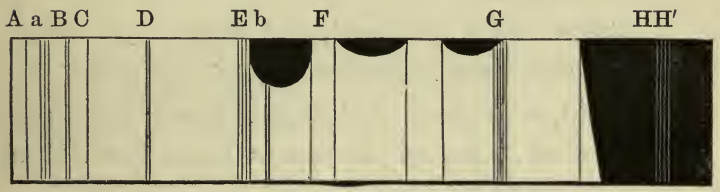
3. *Chloroform extract.*—Digest egg-yelk in chloroform, filter, and allow to stand till clear. The solution has a fine yellow colour, and in the spectroscope shows three bands.



Spectrum of egg luteine in chloroform.

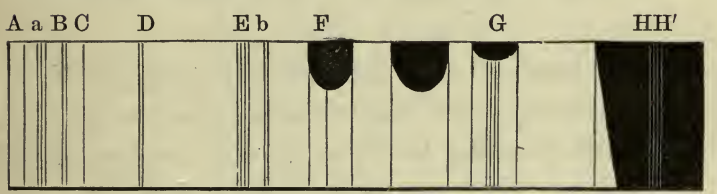
III. *Butyro-luteine.*

1. Butter is digested with chloroform and filtered. Three bands.



Spectrum of butyro-luteine in chloroform.

2. Dried by heat and filtered hot. Three bands, fainter when heated; third only visible when just cooling.

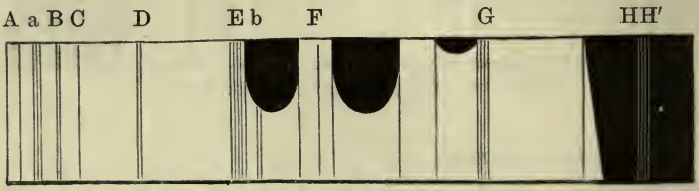


Spectrum of butyro-luteine in fused butter.

IV. *Cysto-luteine.*

1. The yellow fluid contained in an ovarian cyst is examined before the spectroscope with the lime light. Three bands in blue will be ob-

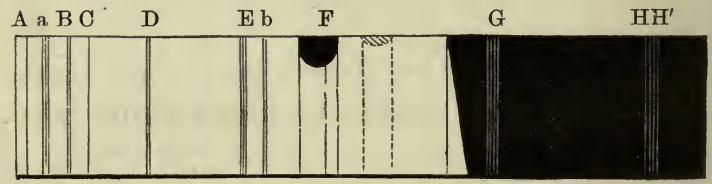
served in the same position as those of the chloroform solution of ovario-luteine.



Spectrum of cysto-luteine.

V. *Sero-luteine.*

1. Allow blood to stand, and decant the serum. Set aside the latter to deposit, pour off the liquid, and filter it through paper repeatedly till clear. Before the spectroscope it will show



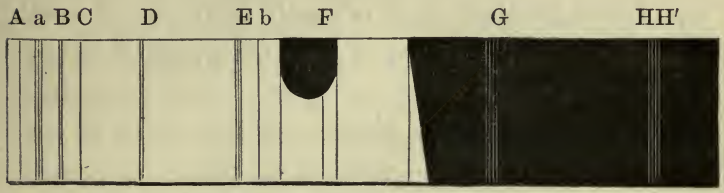
Spectrum of sero-luteine.

the bands of hematocrystalline (*q. v.*), and also (probably) one band and a doubtful second, corresponding to the ovario-luteine bands. If diluted till the blood bands disappear the luteine bands will also become invisible.

VI. *Intestino-luteine*, from Infants.

1. Mix the yellow fæces of sucking infants with alcohol in excess, filter from the flakes of

caseine, and examine the solution with the spectroscope. One band and a doubtful second in the positions of the other luteine bands, will be perceived.



Spectrum of intestino-luteine.

The solution will deposit crystals of *cholesterine*. Examine also the ether and chloroform solutions.

Compare with the above a chloroform extract of dried fæces of adults. It will give no band.

Lymph.—1. Examine under the microscope; white corpuscles and fat globules will be noticed.

2. Allow fresh drawn lymph from a blister, or a fistula of a lymph vessel to stand; it will coagulate. Remove the coagulated threads by beating with a bundle of twigs, and wash them with water. They will be found to consist of *fibrine* (see Fibrine).

3. Strain the liquid and heat it to boiling. A precipitate of *albumen* in flakes will be produced.

4. Filter; to the hot filtrate add a slight excess of dilute sulphuric acid; allow to cool, and shake repeatedly with small portions of ether. Evaporate the ether to dryness, and digest with water. The part

insoluble in water will consist of *fats*. The soluble portion will contain *lactic acid* (*q. v.*).

5. Dry a portion of fresh lymph at 100° C., powder the residue, and burn to a white ash. Examine the ash: it will contain sulphate, phosphate, carbonate, &c., of the alkalies, and a small quantity of earthy salts. Notice prevalence of soda over potash salt.

Melanine.—The black pigment of the eye or of melanotic cancers must be isolated as much as possible, and purified by solution in ammonia and precipitation by hydrochloric acid. It will possess a composition similar to *uromelanine* (*q. v.*), but different properties. A portion will be found to be quite insoluble in any ordinary reagent.

Milk.—1. Milk has a specific gravity 1·018 to 1·045.

2. Test the reaction with litmus paper; the reaction is usually alkaline.

3. Examine some milk under the microscope; it will appear as a clear liquid, in which float the milk globules. These vary in diameter from about 0·0012 to 0·0030 inch.

4. Add a little acetic acid to milk; the globules will become distorted.

5. Shake up some milk with ether; the globules, though they consist of fat, are not dissolved. This is due to the fact that each globule has an envelope which is insoluble in ether.

6. Shake up some milk with caustic potash, which

dissolves the envelope. On now treating with ether the globules will be dissolved.

7. The colostrum (milk secreted during the first two or three days after parturition) is characterised by the presence of granular bodies. Examine under the microscope: the granular bodies will be seen to be composed of irregular aggregations of small fat globules, and united by an albuminous amorphous granular substance. Treat with iodine water; the albumen will be dyed yellow. Potash and acetic acid break up these granular bodies.

8. Evaporate 20 cc. of milk to dryness in a small weighed porcelain or platinum crucible on the water-bath. Dry in an air-bath for several hours at 110° C. and weigh; the increase in the weight of the crucible gives the solid residue in the milk.

9. Ignite the dried residue over a Bunsen or Argand burner; allow to cool and weigh. The difference between this weight and the original weight of the crucible gives the ash of the milk.

Milk contains about 10% solid residue, and 0·1 to 0·5% of ash.

10. Evaporate 50 cc. of milk on a water-bath almost to dryness in a weighed porcelain dish; add acetic acid. Exhaust the residue successively with ether, alcohol, and water. Dry the exhausted residue and weigh; the increase in the weight of the dish gives the caseine in the milk.

11. Evaporate the ethereal extract to dryness in a weighed crucible and weigh; the increase of weight will give the quantity of fat.

12. Add to 100 cc. of milk solution of calcium chloride; the caseine is precipitated; filter; add potash solution to precipitate excess of calcium, and estimate the sugar present in the filtrate with standard solution of potassio-tartrate of copper.

Milk contains 2 to 4% caseine.
,, 1·5 to 4% fat.
,, 4 to 5% sugar (lactose).

Mucine.—1. Dilute mucus with four vols. of water and filter. Digest the insoluble matter (mucine) with a weak solution of potash, filter, neutralise with acetic acid, wash the precipitate with water, alcohol, and ether, and use it for the following reactions.

2. Dry a portion and burn on platinum foil. A white alkaline ash containing calcium phosphate will remain.

3. Fuse with caustic potash, dissolve in water, and add a drop of lead acetate. No black precipitate, showing the absence of sulphur.

4. Mix with cold water and boil. It will gradually dissolve. Add alcohol; the mucine will be precipitated in flakes.

5. Dissolve mucine in a dilute acid or alkali, and add potassium ferrocyanide. No precipitate.

6. Dissolve in glacial acetic acid and boil. Add potassium ferrocyanide; white precipitate.

7. Heat with concentrated nitric acid: the mucine will turn yellow and dissolve.

8. Dissolve in very weak caustic potash, and add basic lead acetate or tannic acid; white precipitate.

9. Dissolve in dilute hydrochloric acid, and add mercuric chloride; only a slight turbidity will ensue.

Muscles.—Striated voluntary and involuntary muscles.—1. Examine under microscope. They will be found to consist of fibres bound together in bundles, and marked with transverse striæ.

2. Free the muscles of a recently-killed animal from blood by injecting through the vessels a one per cent. solution of sodium chloride. They must then be frozen, minced, mixed with four vols. of snow containing a little sodium chloride. The mass will liquefy, and must be quickly filtered at 0°C. The nearly clear filtrate on regaining the ordinary temperature will coagulate. Stir with a rod, and separate the coagulum from the serum.

3. Wash the coagulum with water, alcohol, and ether. It will be found to consist of *myosine* (*q. v.*).

4. Heat the serum to boiling: *albumen* will precipitate in flakes.

5. Extract the cake which remains after the extraction of myosine and albumen by process 2 with pure water, and observe that the red colouring matter *myochrome*, identical with hemato-crystalline, dissolves. Study its properties as prescribed for hemato-crystalline.

6. Expose a piece of fresh muscle to oxygen under a receiver, and observe that oxygen is absorbed and *carbonic acid* evolved.

7. After extraction of the myochrome (5), treat the

residue with dilute hydrochloric acid and obtain *syntonine* (*q. v.*).

8. Extract *kreatine*, *kreatinine*, and *inosic* acid by the processes described under those bodies.

9. Extract *sarkine* and *xanthine* by the processes described under those bodies.

10. Extract *inosite* and *dextrine* by the processes described under those bodies.

11. Extract *kreatylic* acid by a process similar to that prescribed for the extraction of kryptophanic acid from urine.

12. Burn a portion of muscle freed from blood as above. Examine the ash, and observe that potash salts prevail in it.

13. Examine mercantile extract of meat (Liebig's); observe what quantity of it is soluble in alcohol of 80% strength. Burn a quantity, and observe that it leaves about 18% of ash, of which half is potash.

14. Examine brine in which meat has been kept; observe that it contains much albumen, but no red colouring matter.

15. Treat red salted meat, such as ham, with water, and observe that the hemato-crystalline is insoluble in it. Boil with alcohol: the red matter will dissolve, and the solution will show a particular spectrum, differing from the spectra of hemato-crystalline, hematine, or cruentine.

Myeline.—Extract brain or blood-corpuscles by ether, and evaporate the extract. The matter thus obtained has received the name of "*myeline.*" It is a

mixture of cerebric acid, several lecithines, cholesterine, and fats. It is interesting as a microscopic object from its developing peculiar stringy growths when placed in water.

Myosine.—From the muscles of an animal just killed; the plasma or liquid portion is removed by pressure, beaten with a rod while it coagulates, and the coagulated flakes of myosine washed with water. In properties myosine closely resembles fibrine, but is flaky, not fibrous, more transparent in appearance, easily soluble in a 10% solution of sodium chloride, and precipitated on dropping this solution into water.

Nerve.—1. Nerve is similar to brain in constitution. The brain fats which have been found in nearly all parts of the organism are probably due to the presence of minute ramifications of nerves.

2. Examine under microscope. Nerves consist of either fibres or cells. The fibres are generally made up of an outer sheath, a "medullary substance," containing albuminous matter and fats, and a central "axis cylinder" of albuminous matter alone. The medullary substance, and perhaps the axis, coagulate after death. The nerve-cell or vesicle has a nucleus and nucleolus, and differs in composition from the nerve-fibre, being soluble in acetic acid.

3. The substance forming the "axis cylinder" resembles fibrine and myosine, but differs from the former by being insoluble in potassium nitrate, from the latter by not dissolving in dilute acids.

4. The nerve-cells contain a substance resembling caseine.

5. The "sheath" of the nerve-fibres appears to consist of elastic tissue (*q. v.*).

(See *Brain* and *Oleophosphoric Acid.*)

Oleophosphoric Acid (Frémy).—1. The ethereal extract obtained in the preparation of cerebrine (*q. v.*) is evaporated and digested with a small quantity of ether. The solution must be shaken with dilute sulphuric acid to remove soda, then washed with water to remove excess of acid. Distil off the ether, and dissolve the residue in boiling alcohol; on cooling the oleophosphoric acid will be deposited, and must be freed, as far as possible, from oleine by washing with cold absolute alcohol, and from cholesterine by ether, in which the latter is the more soluble.

2. Sodium oleophosphate may also be obtained from muscle, &c., by extracting with cold dilute alcohol, and treating the extract as above.

3. Oleophosphoric acid is a yellowish viscous substance, soluble in ether and in hot alcohol, insoluble in cold absolute alcohol and in water, but swelling up slightly in boiling water from the presence of a little cerebric acid.

4. Long boiling with water or alcohol, especially if acidified, decomposes it into oleine and phosphoric acid, or into oleic and glycerophosphoric acids. The same change takes place in the brain by physiolysis or putrefaction.

5. Alkalies in excess resolve it into phosphate, oleate, and glycerine.

6. Heated on platinum foil it blackens, burns, and leaves a charcoal containing phosphoric acid corresponding to 2% of phosphorus in the original substance. The phosphorus may also be determined by decomposing the compound by fuming nitric acid, when the phosphoric acid is found in the aqueous layer.

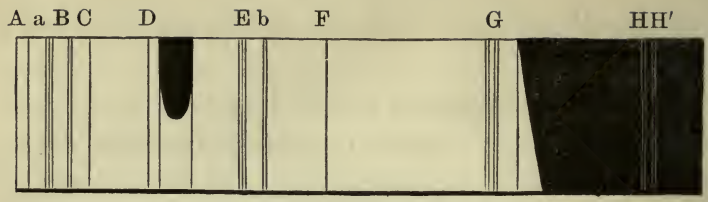
7. Oleophosphoric acid differs from oleine in being insoluble in cold absolute alcohol. It has not yet been obtained pure, nor can it be produced artificially, but a similar compound of *sulphuric acid and oleine* is known.

Omicholic Acid, $C_{15}H_{22}NO_4$ (See *Uromelanine*).—1. Boil Urochrome (*q. v.*) with dilute sulphuric acid until the fluid assumes a dark red colour; add water, reddish brown flakes are precipitated, collect those flakes and extract with boiling alcohol; filter the solution when cool, and concentrate the filtrate and pour into cold water; a red powder is deposited; collect this powder and extract with ether, and filter.

2. Allow the ethereal solution to evaporate spontaneously; the *Omicholic acid* is left as a resinous syrup mixed with *Omicholine*.

3. Treat with ammonia; the omicholic acid is dissolved whilst the omicholine remains.

4. Examine the ethereal solution of omicholic acid with the spectroscope. It has a spectrum showing an absorption band in the green. The ammonia solution has no band.



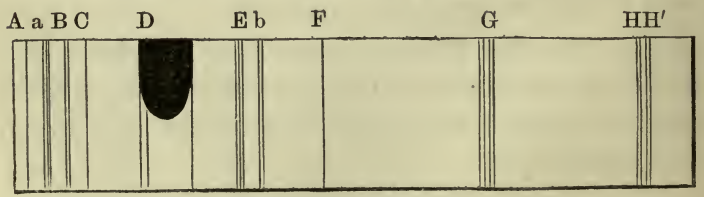
Spectrum of omicholic acid.

5. Heat a very small portion on platinum foil; a very strong urinous odour will be evolved.

6. Examine the ethereal or chloroform solution in concentrated sunlight, and notice a strong green fluorescence.

Omicholine, $C_{22}H_{38}NO_5$ (see *Uromelanine*).—1. The alcohol solution of resins obtained as described under Uromelanine must be concentrated and poured into water. Dry the precipitate, extract with ether, and filter. The portion insoluble in ether is *Uropittine* (*q. v.*).

2. Evaporate the ether, dissolve the residue in a little hot absolute alcohol, and pour into water. Collect the flocculent precipitate and treat with dilute ammonia. *Omicholic acid* will dissolve, while *Omicholine* will remain insoluble.



Spectrum of omicholine.

3. Omicholine is insoluble in cold, slightly soluble in boiling water, insoluble in alkalies when pure, very soluble in alcohol or ether with a red colour.

4. Heat a small portion on platinum foil; a powerful urinous odour will be evolved.

5. Examine the alcohol solution with the spectroscope; a band in green will be observed.

6. Examine with a lens in sunlight; the solution fluoresces green.

Pancreatic Juice.—1. Notice appearance and alkaline reaction.

2. Add tannic or a mineral acid; a white precipitate will be produced.

3. Heat to 72°, a white precipitate will also appear.

4. Add three or four volumes of 94% alcohol, filter, dry the precipitate, dissolve in water, and filter. Digest solution with some starch; the latter will be dissolved and converted into dextrine and sugar. Digest with coagulated albumen; it will dissolve. With fats it will form an emulsion.

5. Evaporate the juice to a low bulk and exhaust with ether. The ethereal solution must be examined for *fats*. The aqueous portion must be tested for *leucine* and *tyrosine* (*q. v.*).

6. Treat pancreatic juice with a little chlorine water; a reddish colour will be produced.

7. Evaporate, burn, and analyse ash.

Pepsine.—1. Treat the glandular layer of a stomach with dilute tribasic phosphoric acid; filter and neutralise

the filtrate with lime-water. Collect the precipitate on a filter, wash and treat with dilute hydrochloric acid. Treat the clear solution with lime-water, collect the precipitate, dissolve in dilute hydrochloric acid, and add, through a thistle funnel reaching to the bottom of the solution a saturated solution of cholesterine in a mixture of one part ether and four alcohol, and shake the whole well. Filter off the precipitate which consists of cholesterine and pepsine, wash, and finally shake up in a bottle with ether; allow to stand, remove the ethereal solution of cholesterine with a syphon and filter the remaining liquid. The filtrate will consist of *a solution of pepsine.*

2. Acidify this solution feebly with hydrochloric acid, add to the solution thus acidified some albumen; it will rapidly be dissolved.

3. Add to the solution tannic or mercuric chloride; no precipitate is formed.

Protagon (Liebreich).—1. The brain of an animal just killed is freed from blood by injecting water till it issues colourless. The substance is then comminuted, shaken with repeated quantities of water and ether (to remove cholesterine and matters soluble in water), and the residue digested with warm (45° C.) 85% alcohol. The alcoholic extract on cooling deposits a flaky precipitate, which must be exhausted with cold ether, and purified by recrystallising from warm alcohol. Pure protagon has the following properties.

2. Light flocculent powder, consisting under the microscope of radiated needles, little soluble in cold,

more soluble in hot, alcohol and ether. In water it swells up to an opalescent solution, which is coagulated by the addition of salts. From the coagulated flakes the salts are almost entirely removed by washing with water, leaving the protagon unchanged.

3. In warm glacial acetic acid it dissolves to a clear solution, which deposits crystalline protagon on cooling.

4. It softens at a temperature of 75° to 80°, and decomposes below 100°. When burned it leaves a dense charcoal difficult of combustion and containing phosphoric acid.

5. By the action of alkalies or boiling dilute HCl it yields *cerebrine* and the decomposition products of *lecithine* (*q.v.*). (See Cerebric Acid.)

Ptyaline.—1. Treat a quantity of saliva first with dilute phosphoric acid and then with lime water. Decant the clear liquid; shake up the precipitate with distilled water and filter. The filtrate is a solution of the active ferment *ptyaline*, which can be precipitated by the addition of alcohol.

2. Treat some starch with the above-mentioned aqueous solution at a temperature of 35°; it will dissolve and be finally converted into dextrine and sugar.

3. The active properties of ptyaline are destroyed by a temperature above 60° C., or by strong acids or alkalies.

4. Treat a little solid ptyaline with nitric acid. No yellow colour will be produced, showing that it is not an albuminoid.

5. Heat with soda lime in a tube: ammonia will be evolved, showing presence of nitrogen.

6. Fuse with caustic potash, dissolve in water and add a drop of lead acetate. A black precipitate will form, showing presence of sulphur.

Pus.—1. Examine under microscope: pus corpuscles, identical with the white corpuscles of the blood, will be noticed. Observe appearance and reaction with litmus paper.

2. Exhaust the pus with twice its volume of boiling 90% alcohol, containing a little hydrochloric acid, evaporate to one third, mix with three or four times its bulk of ether, shake well, and decant or fill the liquid. Examine the solution and the insoluble residue by the methods described under fats; cerebric, oleic, and palmitic acids and cholesterine will be found.

3. Dilute pus with three volumes of water containing a little sodium chloride, and by filtration and decantation separate the corpuscles from the serum.

4. Heat the serum: a precipitate of *albumen* will be formed. Filter: test a portion of the filtrate for *sugar* by the copper test. Evaporate another portion to a low bulk and add nitric acid; a crystalline precipitate of urea nitrate will indicate *urea*. In another portion test for *leucine* (*q.v.*).

5. Digest the corpuscles with a 10% solution of sodium chloride, filter or decant, and mix the solution with a large quantity of distilled water. A flaky precipitate will form of a substance allied to *myosine*. The portion of the corpuscles insoluble in sodium

chloride must be digested with dilute hydrochloric acid, filtered, and the filtrate carefully neutralised with soda. A precipitate of *pyine* will appear (*q.v.*).

6. Distil fresh pus carefully with dilute phosphoric acid. To the distillate add some sodium carbonate to very slight alkaline reaction, evaporate down and test for *volatile acids* (*q.v.*).

7. Evaporate pus to dryness at 100° C. and burn. Examine the *ash*.

Pyine. — 1. Obtain from pus by the method described under *Pus*, 5.

2. Dissolve in dilute caustic potash, and add acetic acid: a precipitate will appear, *insoluble in excess of the acid.*

3. To the solution in potash add hydrochloric acid: a precipitate soluble in excess will be produced.

4. To the solution in dilute hydrochloric acid add potassium ferrocyanide: no precipitate.

5. Treat with nitric acid: it will turn yellow and dissolve.

6. To a solution in very dilute nitric acid add lead acetate or mercuric chloride: a white precipitate will fall.

7. Dissolve in weak hydrochloric acid or caustic potash and boil: no precipitate will ensue.

Pyocyanine.—1. Linen stained with "blue pus" must be extracted with cold water containing a little ammonia, and the evaporated and filtered extract digested with chloroform. Shake the chloroform

solution with very weak sulphuric acid; treat the red aqueous layer with baryum carbonate, and baryta water till it turns blue, filter, shake the filtrate with chloroform, and evaporate the chloroform solution in a current of air. *Pyocyanine* will gradually deposit in crystals or flakes.

2. It is soluble in water, alcohol, and chloroform, but almost insoluble in ether. Acids turn it red; alkalies blue. It is decolorised by chlorine.

3. From the mother liquor of pyocyanine, a yellow substance, *pyoxanthose*, may be obtained.

Saliva.—1. Extract ptyaline by the method described under that body.

2. Distil with a dilute acid (phosphoric or sulphuric): in the distillate ferric chloride will produce a red colour, showing the presence of *sulpho-cyanide* (*q.v.*).

3. Evaporate to dryness and burn. Analyse the ash. It will be found to contain calcium carbonate.

4. If the saliva be turbid, dilute it with four volumes of water, and filter. The insoluble matter will be *mucine*. (See Mucine.)

Sarkosine, $C_3H_7NO_2$.—1. Boil kreatine with baryta water till no more ammonia is given off, filter, add to the filtrate an excess of dilute sulphuric acid, boil, and again filter. Evaporate to a low bulk, mix thoroughly with three or four successive small portions of cold alcohol, decant the alcohol, dissolve the residue in water, boil with baryum carbonate to remove sulphuric

acid, filter, concentrate to a syrup at 100° C., and set aside to crystallise.

2. Sarkosine is very soluble in water, nearly insoluble in alcohol, and insoluble in ether. Notice neutral reaction to litmus of aqueous solution.

3. Add to an aqueous solution cupric acetate: an intense blue colour will be produced. By evaporation dark blue crystals of a double salt may be obtained.

4. In a cold saturated solution of mercuric chloride place a crystal of sarkosine: the liquid will gradually solidify to a network of white needles.

5. To a solution of sarkosine sulphate add lead peroxyde; decomposition with effervescence will take place, and the solution will contain *methylamine*.

6. Dissolve sarkosine in hydrochloric acid, add platinic chloride, and set aside to evaporate. Yellow octahedra of a platinum salt, 2 ($C_3H_7NO_2$, HCl) $PtCl_4$, $2H_2O$, losing water at 100°, will be deposited.

7. Heat sarkosine in a tube; it will melt and sublime without residue.

8. Heat another portion with soda-lime: methylamine (with a smell of ammonia and alkaline reaction) will be evolved.

Succinic acid, $C_4H_6O_4$.—1. Obtain from the fluid in the bladders of echinococci by evaporating to a syrup, adding hydrochloric acid and extracting with ether. The ether solution after distillation leaves succinic acid in crystals.

2. Heat in a tube open at both ends. The acid will sublime in silky needles.

3. It is soluble in water, alcohol, and ether. Dissolve in water, and use for the following reactions.

4. Neutralise with soda, evaporate to crystallisation. Heat a portion of the crystals in a tube; it will blacken, leaving a residue of sodium carbonate mixed with charcoal.

5. Neutralise with ammonia and add ferric chloride. A bulky red-brown precipitate will be formed, easily soluble in mineral acids. Add excess of ammonia, boil, and filter. To the filtrate add baryum chloride and a little alcohol; a white precipitate will fall. Benzoic acid will give no white precipitate under these circumstances.

6. Add lead acetate; a white precipitate will ensue.

Sulphocyanic acid, CNSH.—1. Obtain by distilling large quantities of saliva with sulphuric acid and neutralising the distillate, or evaporate the saliva to dryness and extract with alcohol; evaporate the alcohol and use the extract dissolved in little water. Most sulphocyanides are soluble in water and alcohol.

2. Fuse with caustic potash; ammonium carbonate will be evolved, and will turn red litmus blue. Dissolve the residue in water and add lead acetate; a black precipitate of sulphide will be formed.

3. Heat 100 grammes of dry ammonium sulphocyanide in a flask to 170°C. in an oil bath for two hours. Allow to cool to 100°C., add twice its bulk of boiling water, filter hot, and set aside to crystallise. A network of long fibrous satiny crystals of *sulphur urea* will be formed. Press, purify by recrystallisation

from water and from alcohol, dissolve a small quantity in a very large bulk of water, and apply the following tests.

a. Add a drop of cuprous chloride in hydrochloric acid; a bulky white gelatinous precipitate will appear.

b. Add mercuric nitrate; an amorphous white precipitate will fall.

c. Add cupric sulphate; a precipitate of white microscopic prisms will gradually form, redissolved by heat and depositing again in oily drops or in crystals on cooling, giving by long boiling a black precipitate of sulphide.

d. Add ferric chloride; no reaction if the urea be pure.

4. Boil a sulphocyanide with nitric acid; a yellow precipitate of persulphocyanogen will be produced.

5. To a sulphocyanide solution add ferric chloride; an intense red colour will be produced. Add a fragment of pure zinc, and suspend over it a slip of paper moistened with lead acetate. The paper will be blackened by the evolution of H_2S.

6. Add solution of cuprous chloride in hydrochloric acid; a white granular precipitate will appear.

Sweat.—1. Notice appearance, smell, acid reaction, and presence of epidermic scales.

2. Distil down to a third or fourth with a little dilute sulphuric acid. Examine the distillate for *volatile acids* (*q. v.*). Digest the residue in the retort with ether.

3. Evaporate the ethereal solution freed from sulphuric acid by digestion with carbonate of baryta, and exhaust with water. The aqueous solution freed from baryta by cautious precipitation with sulphuric acid examine for *lactic acid* and *urea* (*q. v.*). The insoluble portion will consist of *fats* (*q. v.*).

4. Examine the portion insoluble in ether for urea, *uric acid*, *sugar*, and *sudoric acid*.

5. Heat a little of the sweat to boiling; a flaky precipitate of *albumen* will, in some rare cases, appear.

6. Evaporate to dryness, burn, and analyse ash.

Synovia.—1. Notice appearance and alkaline reaction.

2. Dilute with two volumes of water, add acetic acid till very slightly acid, and boil. A precipitate of *albumen* will be formed.

3. Evaporate to a low bulk at 100°C. and exhaust with ether. The ether on evaporation will leave a residue of *fat*.

4. Evaporate to dryness and burn. Analyse ash.

Syntonine or *Muscle-fibrine*.—1. Wash comminuted muscle with cold water till free from albumen, digest with very dilute hydrochloric acid, strain, neutralise the solution with sodium carbonate, and wash with water the precipitate of syntonine.

2. The reactions are similar to those of fibrine, except that it is more soluble in dilute hydrochloric acid.

Taurine, $C_2H_7NSO_3$.—1. Mix ox bile with hydrochloric acid, filter the liquid from the precipitate which will fall, evaporate the filtrate till it separates into a viscous resin and a watery liquid; pour off the liquid and rinse the resin with water. Unite the two liquids, and evaporate to a small bulk; allow to crystallise; taurine and sodium chloride crystallise out; the taurine is separated by picking out the crystals, which can be purified by recrystallisation.

2. Taurine crystallises in large transparent glassy crystals, which when heated first melt and then blacken. It is moderately soluble in water, nearly insoluble in alcohol.

3. Melt some taurine with caustic potash; treat the residue with dilute sulphuric, holding a slip of paper moistened with lead acetate solution over the mass; sulphuretted hydrogen is evolved, turning the lead paper black; sulphurous acid is also evolved, and sulphur is left as a residue. This proves the existence of sulphur in taurine.

Taurocholic acid, $C_{26}H_{45}NSO_7$.—1. Extract some dog's bile with alcohol, decolorise by animal charcoal, and evaporate the extract to dryness. Dissolve the residue in a small quantity of absolute alcohol and ether; on standing in the cold *sodium taurocholate* is precipitated *in crystals*.

2. Precipitate ox bile with neutral lead acetate, and filter. Precipitate with basic lead acetate, and wash the collected precipitate; dissolve it in boiling alcohol,

and decompose with hydrothion. The solution on evaporation leaves *amorphous taurocholic acid.*

3. Taurocholate of sodium is not precipitated by acids but by caustic potash. Its solution in water froths like soap-lather. It is not precipitated by neutral lead acetate.

4. By boiling with hydrochloric acid it is decomposed, yielding *taurine*, *choloidic acid*, and *dyslysine.*

5. By boiling with excess of caustic baryta it is also decomposed, yielding taurine and *cholic* acid, which latter remains in combination with the baryum.

6. On fusion with caustic potash taurocholic acid and taurocholates behave like *taurine* (3), forming sulphides, which are decomposed by acids under evolution of hydrothion.

7. Determine the quantity of taurocholic acid by the process *Bile*, 14.

Trimethylamine, C_3H_9N.—1. Distil urine with lime; saturate the alkaline distillate with sulphuric acid, evaporate to dryness, and extract with absolute alcohol. Trimethylamine sulphate if present dissolves, and may be purified by recrystallisation, and the trimethylamine liberated by distillation with potash.

2. To a little of the sulphate add calcium hydrate in powder, and warm. Trimethylamine will be disengaged, with a peculiar odour of bad fish.

Tyrosine, $C_9H_{11}NO_3$.—1. Boil horn shavings with twice their weight of dilute sulphuric acid (one part of concentrated sulphuric acid and four parts of water)

for four hours, renewing the water as it evaporates. Dilute with water, add an excess of milk of lime, and strain, exhausting the residue with boiling water. Evaporate the filtrate and extracts to about two thirds of the bulk of the dilute sulphuric acid. Neutralise with sulphuric acid, and allow the whole to stand. Impure tyrosine crystallises out.

2. Purify and decolorise by dissolving the tyrosine in a little hydrochloric acid and boiling the solution with animal charcoal. Filter and precipitate the tyrosine by adding acetate of soda solution. Crystallise the tyrosine by dissolving in a little hot strong ammonia, and allowing to cool, when the tyrosine will separate out in white acicular tufts.

3. Tyrosine is moderately soluble in boiling water, very sparingly soluble in cold water, almost insoluble in alcohol, it is easily soluble in hydrochloric acid, ammonia, &c.

4. To a small portion of tyrosine add a little Nordhausen sulphuric acid; the tyrosine dissolves, let stand for some time; dilute rapidly with water, neutralise the solution with baryum carbonate, filter, add to the filtrate some neutral solution of ferric chloride when a violet colour is produced.

5. Heat some tyrosine in a glass tube, it decomposes, evolving a strong agreeable smell.

6. Add to an aqueous solution of tyrosine a little mercuric nitrate with mercurous nitrate, a pink colour and red precipitate will be produced on warming.

Urea, CH_4N_2O.—1. Evaporate urine to dryness on

a water-bath; exhaust the residue with hot alcohol; evaporate the solution to dryness; take up with absolute alcohol; evaporate the solution to dryness, when crystals of urea will be obtained.

2. Evaporate a solution of cyanate of ammonium to dryness on a water-bath; crystals of urea will be obtained.

3. Urea is very soluble in water, and alcohol, nearly insoluble in ether; on heating, it melts and then decomposes. Examine the crystals under the microscope.

4. Take a small quantity of urea, dissolve in water, add a saturated solution of sodium carbonate, place the whole in a flask, and distil through a Liebig's condenser previously well washed with distilled water; collect the distillate; on adding some Nessler test-solution a dark-brown colour will be immediately formed, proving the decomposition of the urea into ammonia and carbonic acid.

5. Add to an aqueous solution of urea a solution of nitrate of mercury; a white precipitate of mercuric oxyde and nitrate of urea falls.

6. Half fill a test-tube with an aqueous solution of urea, fill up the tube with a solution of hypochlorite, close the test-tube with the thumb, invert the whole once or twice to mix the contents thoroughly, and, finally, invert the tube under the surface of a saturated aqueous solution of salt. Allow to stand; bubbles of gas will soon be disengaged, and collect in the upper part of the test-tube. This gas is nitrogen formed from the decomposition of the urea.

7. Add to a concentrated solution of urea nitric acid; crystals of urea nitrate will immediately form. Add oxalic acid to urea, and the oxalate will be deposited.

Estimation of urea.—1. Dissolve in a beaker 100 grammes of pure mercury in about 500 grammes of pure nitric acid; add a further quantity of nitric acid in drops, gently shaking occasionally until no red vapours are evolved either on the addition of nitric acid or on shaking; then evaporate the solution at a gentle heat until it is a colourless syrup. Care must be taken that none of the liquid is lost by spurting.

2. Dilute the solution to exactly 1400 c.c., adding a little nitric acid to prevent the formation of an insoluble basic salt. This forms the standard solution of nitrate of mercury, each cubic centimetre of which represents a centigramme of urea. To be certain of its strength a test experiment should be made by estimating a known quantity—about two decigrammes—of pure urea as described hereafter.

3. Prepare cold saturated solutions of baryta water and baryum nitrate; mix two volumes of baryta water with one volume of nitrate of baryum solution. This mixture forms the baryta solution used in the analysis.

4. On a glass plate under which is a piece of white filter paper, place a number of small drops of carbonate of soda.

5. Add to 30 c.c. of urine 15 c.c. of the baryta solution; mix well with a stirring-rod, and filter through

a dry filter paper. Measure off 15 c.c. of the filtrate (=10 c.c. of urine) into a small beaker, add the standard nitrate of mercury solution from a burette or other graduated vessel until a drop of the mixture when added to a drop of carbonate of soda solution on the glass plate produces a distinct yellow colour in two seconds. When this colour appears read off the number of cubic centimetres used; each cubic centimetre required indicates 1 centigramme of urea in the 10 c.c. of urine. Thus, if 25 c.c. of mercury solution were used there would be 25 centigrammes or 0·25 grm. in 10 c.c. or 25 grm. in the litre.

Uric acid, $C_5H_4N_4O_3$.—1. Obtain from human urine by adding to it $\frac{1}{20}$ hydrochloric acid, let stand in a warm place at first, afterwards in the cold, and collect the precipitate of crystallised coloured acid. Purify as described in 2.

2. Obtain from excrements of serpents by dissolving them in hot caustic soda ley, boiling to expel ammonia, precipitating urate by a current of carbonic acid, dissolving the urate in caustic soda, and pouring this solution in hot dilute hydrochloric acid. White uric acid in crystals is deposited.

3. Observe its forms of crystallisation under the microscope, and make yourself acquainted with the principal typical forms (rhombic prisms and plates) which the acid assumes, particularly when it is deposited spontaneously in the urinary passages or in the urine after emission.

4. Dissolve uric acid in boiling water, and observe that a deposit ensues on cooling. Dissolve it in alkalies, and observe that the salts with excess of alkali are much more soluble than the salts with excess of acid.

5. Dissolve one part of uric acid in four parts of nitric acid of 1·42 sp. gr. The acid will dissolve under evolution of carbonic acid, nitrogen and nitrous acid, and on cooling *alloxan* (see p. 67) will be deposited.

6. Evaporate the solution of uric in nitric acid to dryness on the water bath, and allow the vapour of ammonia to touch the residue. A purple colour of *murexide* will be produced.

7. To uric acid made into a pap with water add gradually lead peroxide, and keep the mixture near the boiling point. The lead is transformed into oxalate, carbonic acid is evolved with effervescence, the filtered fluid deposits crystals of *allantoine* on cooling, and the mother liquid contains urea.

8. Heat uric acid in closed tubes with concentrated hydrochloric or hydriodic acid, and obtain *glykokoll* by decomposition of the resulting salt as described under that substance.

9. Examine the urates spontaneously deposited on cooling from healthy or morbid urine; collect them on a filter, and observe that when washed with water crystals of uric acid gradually form in them, but that this formation does not take place when the washing is effected with spirit. Determine the quantity of bases contained in them, which are mostly a mixture of potash, soda, and ammonia, and notice that their collective

basic value only amounts to about one half of what the whole of the uric acid would require to be in the condition of acid urate. These deposits are therefore *hyper-acid urates.*

Urine, systematic analysis.—Test the action of the urine with litmus.

I. It is acid and has no sediment, proceed to 2.

II. It is acid and has a sediment; pour off the clear liquid, filtering, if necessary, and proceed to analyse the filtrate according to 2. Examine the sediment dry.

1. Heat a sample of urine to boiling after the addition of some acetic acid. A coagulum forms, which does not disappear on the addition of nitric acid: *albumen.*

   Boil some quantity (500 c.c.) of the urine with acetic acid; filter off the coagulated albumen and treat the filtrate as under 2.

   a. The coagulum is white, *pure albumen.*

   b. The coagulum is greenish: albumen, probably coloured by bile.

   c. The coagulum is brownish-red: probably from blood; wash and dry the coagulum; boil with alcohol containing a little sulphuric acid; if the filtrate is reddish examine with spectroscope for acid hematine or evaporate to dryness, ignite, moisten the ash with a drop or two of concentrated hydrochloric

acid, dilute with a little water, filter the solution through a small filter, and add to the filtrate a little potassium sulphocyanide; a red colour confirms the presence of blood.

2. Take 4 to 500 c.c. of the clear urine filtered from coagulated albumen or sediment; evaporate in a porcelain dish or a water-bath to a thick syrup; divide the syrup into two parts, one equal to one 3rd the other two 3rds of the whole.
   a. Extract the 3rd with strong alcohol; filter, and examine the filtrate.
      1. Evaporate a small portion nearly to dryness and add a little nitric or oxalic acid, and observe the crystalline forms of *urea nitrate or oxalate.*
      2. Precipitate the larger portion with a few drops of milk of lime and calcium chloride solution and filter; concentrate the filtrate on the water-bath to 10-12 c.c. Transfer to a beaker, add one-half c.c. of strong alcoholic solution of zinc chloride, stir well and allow to stand; *kreatinine chloride of zinc* crystallises out in warty grains.
   b. Acidify the two-thirds with hydrochloric acid, and extract with ether. Evaporate the ethereal solution and examine the residue for *hippuric acid.*
      1. The filtrate will contain *earthy phosphate* and other salts; add ammonia; the *earthy* phosphates will be precipitated.

2. The insoluble residue consists of *mucus* and *uric acid*. Wash off the filter into a test tube, add one or two drops of caustic soda, warm and filter. The insoluble residue is *mucus*. The filtrate contains uric acid and hydrochloric acid; the *Uric acid* separates out in crystals; collect and examine under the microscope, also verify by applying the murexide test, the presence of *uric acid*.

3. The urine is brown or green; froths on shaking; colours a small piece of immersed filter-paper yellow or green; probable presence of *bile matter*.

Place some of the urine upon a white plate and drop in a little strong nitric acid containing some nitrous, without shaking. The fluid turns successively green, blue, violet, and brown; presence of a derivate of colouring matter of the bile.

To a second portion add some lead acetate in solution; collect the precipitate, wash, dry, and boil the dried precipitate with alcohol, to which a little sulphuric acid has been added, filter; the filtrate is green from *biliprasine*.

Evaporate a third portion of 3 to 500 c.c. on the water-bath, extract with alcohol; search for biliary acids, tauro- and glykocholic.

4. Take 1 c.c. of urine, dilute it with 4 to 5 c. c. of water, add ½ a c. c. of caustic soda, and one drop of a very dilute solution of copper sulphate; boil; a red granular precipitate of suboxyde of copper indicates the presence of *sugar*.

5. Immerse in the urine a piece of filter-paper moistened with acetate of lead solution, if the lead paper turns brown or black *sulphuretted hydrogen* is present.

6. Evaporate 40 to 50 c. c. of the urine to dryness, ignite the residue at a moderate heat till all the charcoal has been burnt off; boil the residue with water and filter.

a.—1. Acidify a portion *of the filtrate* with hydrochloric acid; add baryum chloride; a white precipitate proves the presence of *sulphuric acid.*

2. Acidify a second portion with nitric acid, add a drop of silver nitrate; a white curdy precipitate indicates *hydrochloric acid.*

3. Acidify a third portion with acetic acid, add a little ferric chloride solution, a yellowish-white, gelatinous precipitate indicates *phosphoric acid.*

4. Evaporate the rest to dryness; take up a small portion on the end of a platinum wire and expose in a Bunsen or spirit lamp

flame; a vivid yellow colour proves the presence of *sodium*.

5. Dissolve a portion in a little water, add a drop or two of solution of platinic chloride; a yellow crystalline precipitate indicates *potassium*.

b. Boil the residue insoluble in water with a little dilute hydrochloric acid, filter.

1. Boil a portion with a drop of nitric acid, add some potassium sulphocyanide solution; a deep red colour proves presence of *iron*.

2. Mix the rest with an excess of sodium acetate, add an excess of ammonium oxalate; a white precipitate proves the presence of *calcium*.

3. Filter off the lime precipitate, add to the filtrate ammonia; a white crystalline precipitate indicates the presence of *phosphate of magnesia*.

7. Add to 50 or 100 c. c. of the fresh urine contained in a flask, a little milk of lime, mix and cork loosely, suspending a moistened red litmus paper between the cork and the side of the flask. If the paper turns blue the presence of *ammonia* is proved.

8. Distil some urine with sulphuric acid, add to the distillate a little red fuming nitric acid, and then shake up with a drop of

carbon disulphide, which, if *iodine* be present, will be coloured pink.

For acetic, benzoic and kryptophanic acid, and for urochrome and its products, see separate articles.

Examination of the Sediment.—Allow any sediment to deposit at the bottom of a conical glass. Pour off as much of the liquid as possible, then take up a little sediment with a pipette, place on a glass slide, and examine with the microscope.

A. The urine is acid.

I. The whole of the sediment seems amorphous.

1. On gently warming the whole dissolves: *urates;* confirm by adding a drop of hydrochloric acid; leave half an hour, when, if uric acid be present, it will have crystallised out in rhombic tables.

Also confirm by the murexide test.

2. The sediment does not dissolve on warming, but dissolves in a drop of acetic acid without effervescence; presence of *calcium phosphate.*

3. Glistening drops appear in the sediment and disappear on the addition of ether: *fat globules.*

II. The sediment contains well-formed crystals.

1. Small, glistening, transparent octohedra, insoluble in acetic acid: *calcium oxalate.*

2. 4-sided tables or 6-sided rhombic plates often appearing grouped in bunches of spindle-shaped crystals : *uric acid ;* confirm by the murexide test.
3. Regular 6-sided tables soluble in hydrochloric acid and ammonia, which char on heating. Boil with caustic soda containing a drop of very dilute acetate of lead ; a black precipitate of sulphide of lead confirms the presence of *cystine.*
4. Wedge-shaped prismatic crystals, some separate, some united, to form a cross : *calcium phosphate.*
5. Greenish-brown grains with a radiating crystalline structure : *tyrosine.*
6. Needles or rhombic prisms easily soluble on warming : *hippuric acid.*

III. The sediment contains organised bodies.

1. Twisted fringy bundles, forming points, grains, &c. : *mucus.*
2. Contracted granular bodies, often united into a scale pavement-like mass : *mucus corpuscles.*
3. Circular biconcave disks, mostly yellowish, which swell up and more or less completely dissolve in acetic acid : *blood-corpuscles ;* confirm by spectroscopic reactions.
4. Round, pale, faintly-granular vesicles, of different sizes, which swell up considerably in acetic acid, lose their outward granular sur-

face, and allow an inner nucleus of different form to be seen : *pus.*

5. Cylindrical masses with small vesicles, often mixed with blood- and pus-corpuscles : so-called *casts of the tubules.*
   a. Casts whose roundish nuclei are clearly visible through a delicate surrounding mass : *epithelial casts of Bellini's tubes*, mostly accompanied by the nucleated, epithelial cells of ureters and kidneys.
   b. Solid cylinders of thick, granular, nucleated nature are *granular renal casts*, and often contain blood- and pus-corpuscles with fat globules, crystals of calcium oxalate.
   c. Pale, transparent, solid cylinders, only seen with great difficulty : *hyaloid casts.*
6. Epithelial cells.
   a. Pavement epithelium.
   b. Epithelial tubes.
7. Fermentation and thread fibres.
8. Short fine rods, threads, or square lumps, moving about in an undulating manner : Vibrios, Spermatozoa, Sarcina ventriculi.

B. The urine is alkaline.

I. Crystalline sediment.

1. Rhombic vertical prisms soluble in acetic acid; on mixing with a little milk of lime ammonia is evolved : *ammonio-magnesian phosphate.*

2. Wedge-shaped opaque masses giving the murexide reaction : *ammonia urate.*

II. Sediment is amorphous, usually *calcium phosphate.*

III. Sediment contains organised bodies, see above under A III.

Urine Oil or Essential Oil of Urine.—1. The distillate from urine with sulphuric or phosphoric acid (*see Uromelanine*) must be neutralised with sodium carbonate, evaporated to a rather low bulk, and extracted repeatedly with small quantities of ether.

2. The aqueous solution contains salts of *volatile acids* (*q. v.*).

3. Distil off the ether from the ethereal solution ; a residue of *essential oil* will remain, with a yellowish colour and a powerful urinous smell.

4. It is little soluble in water, becoming milky when mixed with it, soluble in ether and alcohol.

5. Warm with mercuric nitrate solution; a purple colour will be produced.

6. Boil with silver nitrate ; no reduction will take place.

Urochrome.—1. Fresh urine is treated with excess of milk of lime or baryta, allowed to stand, and filtered. To the filtrate lead acetate with a little ammonia is added till colourless, and the precipitate well washed and digested with cold dilute sulphuric acid till a filtered sample shows an excess of sulphuric acid when tested with baryum

chloride and hydrochloric acid. At this point filter the whole, shake the filtrate with baryum carbonate to remove the sulphuric acid, add a little baryta water, pass carbonic acid through the liquid and filter again. Precipitate the solution with mercuric acetate, wash the precipitate of urochrome mercury very thoroughly with cold and hot water, decompose it by sulphuretted hydrogen, filter, shake the filtrate with a little fresh silver oxyde to remove hydrochloric or kryptophanic acid, filter; again decompose by sulphuretted hydrogen, and evaporate the filtrate to dryness in vacuo over sulphuric acid. A yellow uncrystallisable mass of urochrome remains.

2. Urochrome is easily soluble in water with a yellow colour, very little soluble in alcohol, soluble in ether, and can thereby be separated from kryptophanic acid, which is insoluble in ether.

3. To the solution in water add lead or mercuric acetate: a flaky yellowish precipitate will fall.

4. Add silver nitrate: a gelatinous precipitate soluble in nitric acid, will form.

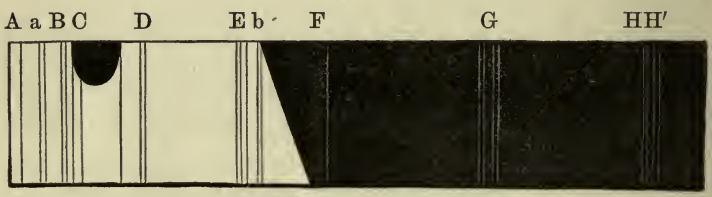
5. Add mercuric nitrate: a white precipitate, pale flesh-coloured on boiling, will be produced.

6. The aqueous solution on standing becomes red and deposits resinous flakes, containing uromelanine, uropittine, omicholine, and omicholic acid (*q.v*). This decomposition is hastened by boiling and by acids.

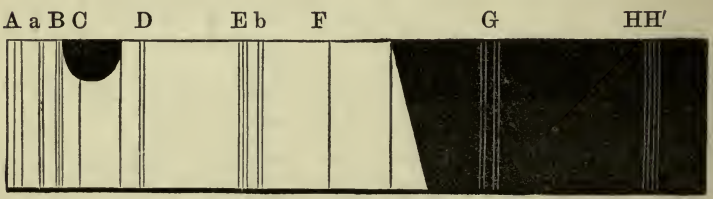
7. Treat acidified extract of urine by ether, and distil the latter. Precipitate the residue by basic lead acetate, decompose by hydrothion, and treat urochrome as above.

8. Separate kryptophanic acid by dissolving its lead-salt in excess of lead acetate, in which urochrome lead is insoluble.

Urocyanine.—Urine from cholera patients in the early stage of reaction, is cautiously boiled with nitric acid. A blue colour is often produced, and a blue deposit sometimes formed. The latter is soluble in alcohol, forming a dichroic purple blue solution giving, before the spectroscope, a broad absorption band in yellow and green.



Spectrum of choleraic urocyanine.



Spectrum of choleraic urorubine.

Uromelanine, $C_{36}H_{43}N_7O_{10}$.—1. Evaporate fresh urine to one tenth, filter, evaporate the filtrate to a syrup, and set aside to crystallise. Decant the mother liquor, dilute with one volume of water, and treat with

calcined magnesia till a filtered sample is free from phosphoric acid. Filter the whole, and to the solution add some concentrated sulphuric acid drop by drop, shaking well, until strongly acid. Filter and distil in a retort for six hours, replacing the water as it evaporates, and adding more sulphuric acid if necessary. The distillate will contain *volatile acids* (*q. v.*) and *essential oil* (*q. v.*)

2. The residue in the retort must be mixed with two volumes of water and allowed to stand. A brown-red resinous precipitate will be formed. Filter.

3. Evaporate down the filtrate, again precipitate with water and filter, and again concentrate and allow to stand; crystals of *hippuric acid* will probably be deposited (*q. v.*).

4. The resinous precipitate is well washed with cold, then with boiling, water, and boiled with 90% alcohol. *Omicholine*, *omicholic acid*, and *uropittine* (*q. v.*) dissolve in the alcohol, while *uromelanine* remains as an insoluble black powder.

5. Wash the uromelanine with boiling alcohol, then with water, dissolve in very weak caustic potash, filter, acidify with dilute sulphuric acid, wash the precipitate with water and hot alcohol, and dry.

6. Uromelanine is insoluble in water and dilute acids, very slightly soluble with a red colour in alcohol, extremely soluble with a brown-black colour in alkalies.

7. Dissolve in acetic acid, and add to the solution mercuric nitrate. A red precipitate will appear.

8. Dissolve in concentrated sulphuric acid, mix the

red solution with water. The uromelanine will be completely precipitated, leaving the liquid colourless. Allow another portion of the red solution to stand some hours, then mix with water; a precipitate will be produced, but the liquid will be coloured.

9. Distil dry uromelanine: a neutral oily distillate will pass over, containing no aniline. Add a drop of mercuric nitrate; a red colour and precipitate will be produced.

Uropittine.—1. The matter insoluble in ether (see Omicholine) must be boiled with absolute alcohol and filtered; the filtrate on cooling will deposit uropittine in granules, which may be purified by again dissolving in alcohol and cooling.

2. Uropittine is a brown resin, fusing in hot water but insoluble in it, slightly soluble in alcohol, insoluble in ether.

3. It is more soluble in boiling than in cold alcohol, and is obtained as a resin by evaporation, or as a powder by precipitation with ether or with water.

4. Dissolve in little ammonia, and add calcium-chloride: a reddish-brown precipitate of uropittine-calcium falls down.

Xanthine, $C_5H_4N_4O_2$.—1. *From Calculi.* Dissolve a small portion of a xanthine calculus in caustic potash, pass carbonic acid through the solution, and wash the precipitate with water.

2. *From Urine.*—Precipitate with baryta water, filter, evaporate to a syrup, and set aside to crystallise. Remove the crystals, dilute the mother liquid, and boil with cupric acetate. Wash the precipitate thereby produced, dissolve in warm nitric acid, reprecipitate with silver nitrate, wash the precipitate, and crystallise it from dilute nitric acid. Wash the crystals with ammoniacal silver solution, decompose by sulphuretted hydrogen, filter, evaporate to a low bulk, and wash with a little water the deposit of *xanthine.*

3. *From Flesh, &c.*—Extract the pounded substance successively with warm alcohol and water, evaporate the mixed extracts and filter, precipitate the filtrate with neutral and basic lead and with mercuric acetate. Treat the precipitates by the two latter with hydrothion in water, filter, and evaporate. Sarkine and xanthine will be deposited; separate by crystallising from hydrochloric acid; the first crystals will be *xanthine hydrochlorate*, while the mother liquid will contain *sarkine* (*q. v.*). Dissolve the former in caustic potash, pass carbonic acid in excess through the solution, and wash the precipitate of *xanthine.*

4. Xanthine is almost insoluble in water, insoluble in alcohol and ether. From hot water it separates inwhite flocks. An aqueous solution rapidly decomposes. It is nearly insoluble in hydrochloric acid.

5. Heat a little dry xanthine in a tube; it will evolve ammonium carbonate and cyanide, and a fetid oil.

6. Dissolve xanthine in hot ammonia; on cooling

crystals of a compound of xanthine and ammonia will be formed.

7. Dissolve xanthine in hot hydrochloric acid. When cool add platinic chloride; a yellow crystalline precipitate of a double salt will ensue.

8. To a *dilute* solution of xanthine in nitric acid, add silver nitrate; no precipitate will be produced (see hypoxanthine).

9. Xanthine is soluble in concentrated sulphuric acid, and the solution is not precipitated by water.

10. To a solution of xanthine in water add mercuric chloride or cupric acetate; a precipitate, white in the first case, green in the second, will be formed.

11. Dissolve a very little sodium in mercury, and place in an aqueous solution of xanthine; the latter will be gradually reduced to *hypoxanthine*.

ALPHABETICAL INDEX.

Acetic acid, 61
Albumen of eggs, 63
„ serum, 33, 83
Alcohol, 65
Allantoine, 56, 67
Alloxan, 59, 67
Amyloid substance, 5, 69
Animal quinoidine, 69

Beef tea, 37
Benzoic acid, 70
Biliary function, 14
Bile, 16, 19, 21, 71
Bilifuscine, 18, 75
Bilirubine, 18, 76
Biliverdine, 18, 77
Blood, system. Analysis, 79
„ corpuscles, 26, 30, 35
Bones, 46
Brain, 40, 85
Breath, 57
Butyric acid, 90
Butyro-luteine, 149

Calculi, biliary, 91
„ intestinal, 94
„ prostatic, 94
„ urinary, 94
Cartilage, 45
Caseine, 97
Cerebric acid, 28, 41, 98
Cerebrine, 41, 99, 100
Cholera, blood in, 31
Cholesterine, 18, 42, 102
Cholic acid, 17, 102
Choline, 18, 41, 141
Cholocyanine, 78
Cholothalline, 78
Chondrine, 103
Chyle, 26, 105
Chyme, 11, 106
Connective tissue, 43, 108
Cornea, 45
Corpora lutea, 144
Cruentine, 108
Cystine, 111
Cysto-luteine, 149

Dentine and enamel, 47
Dextrine, 5, 112
Diabetes, 9
Diabetic sugar or Glykose, 125
Diastase, 4
Digestion, 1, 10

Elastic tissue, 113
Emulsine or synaptase, 4
Excretine, 113
Excretolic acid, 114

Fæces, 58, 114
Fats, 22, 115

Fatty degeneration, 25
Fibrine, 32, 117
Fluopittine and fluorescentine, 118
Formic acid, 61, 118

Gases in intestines, 59
Gastric juice, 10, 119
Gelatine, 43, 120
Globuline, 121
Glycerophosphoric acid, 41, 90
Glykocholic acid, 17, 121
Glykogen, 7, 122
Glykogeny, 3.
Glykokoll, 18
Glykose or glucose, 125
Guanine, 127

Hair, 128
Hematic acid, 30
Hematine, 28, 128
Hemato-crystalline, 27, 80
Hippuric acid, 55, 130
Hyaline, 45
Hypoxanthine, 131

Inosic acid, 132
Inosite, 133
Intestino-luteine, 150

Kidneys, 54
Kreatine, 55, 134
Kreatinine, 55, 134
Kreatylic acid, 156
Kryptophanic acid, 55, 136

Lactic acid, 47, 138
Lecithine, 41, 139
Leucine and leucic acid, 142, 144
Liver, 52
Luteine, 144
Lymph, 151

Margaric acid, 41
Melanine, 152
Milk, 152
Mucine, 154
Muscles, 36, 39, 155
Myeline, 43, 156
Myochrome, 37
Myosine, 36, 157

Nerves, 40, 157
Neurine, 19, 41

Oleic acid, 41
Oleophosphoric acid, 158
Omicholic acid, 55, 159
Omicholine, 55, 160
Osseine, 46, 120
Osteomalacia, 47
Ovaries, 52

Pancreas, 52
Pancreatic juice, 13, 21, 161
Paraglobuline, 29, 33, 84
Paraphanic acid, 56
Peptones, 11, 20
Pigment, 44
Protagon, 41, 162
Ptyaline, 3, 163
Pus and Pyine, 48, 164, 165
Pyæmia, 49
Pyocyanine, 49, 165

Renculi, 51
Rhachitis, 47

Saliva, 1, 166
Sarkosine, 55, 166
Sero-leutine, 150
Serum, 33
Starch, 4
Spleen, 49
Stomach digestion, 14
Succinic acid, 167
Sulphocyanic acid, 168
Sulphocyanides (rhodanates), 3

Sweat, 56, 169
Synovia, 45, 170
Syntonine, 36, 170

Taurine, 17, 171
Taurocholic acid, 17, 171
Teeth, 47
Thymus, 50
Thyroid, 51
Trimethylamine, 172
Tyrosine, 172

Urea, 55, 173
„ estimation of, 175
Urine, systematic analysis of, 178
„ sediments in, 183
„ oil, 186
Urochrome, 186
Urocyanine, 188
Uromelanine, 54, 188
Uropittine, 54, 190

Xanthine, 190

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